

What kind of drugs should we develop

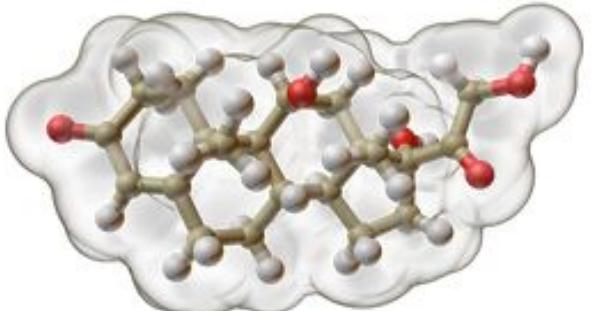
*Mathematical and Computational Biology in Drug Discovery
(MCBDD) Module III*

*Dr. Jitao David Zhang
April 2021*

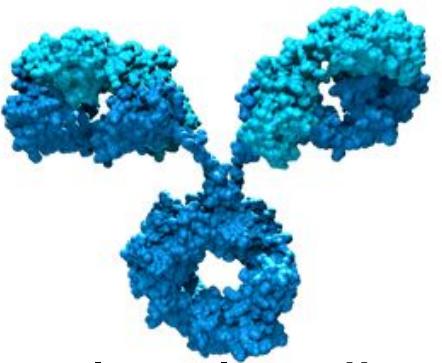
Overview

- Essentials of modalities
 - Small molecules: classical, protein degrader, RNA modulator
 - Large molecules: classical, DUTA-Fabs, protein design
 - Antisense oligonucleotides: siRNA, shRNA, ASO
 - Gene and cell therapy
- Three case studies:
 - Success stories:
 - [Small molecules] SMA (Evrysdi/Risdiplam and Nusinersen)
 - [Antisense] patisiran ([KEGG DRUG](#)) and givosiran ([DrugBank](#), [structure available at EMA](#))
 - [Offline read] mRNA vaccine (MIT Technology Review)
 - Turning failure into successes: [Multispecific drugs] Thalidomide, PROTAC, degraders
 - [Antibody] Cancer immunotherapy (CTLA4, PD1)
 - [Gene and Cell therapy] CAR-T
 - Challenges
 - [Antisense] HTT (Tominersen)
 - Difference between genetic and enzymatic inhibition

A zoo of modalities



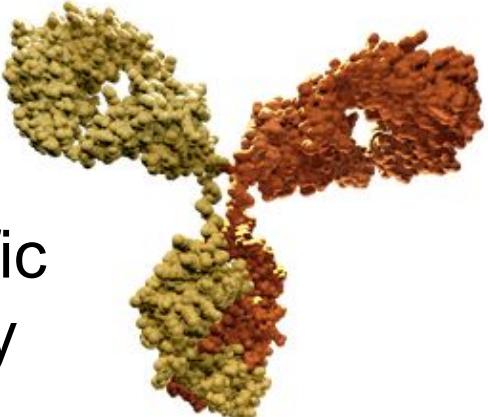
Small molecule



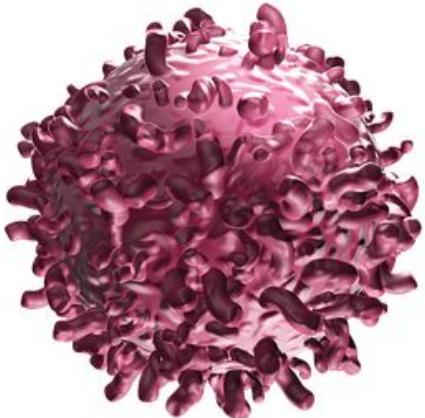
Monoclonal antibody



RNA inference



Bispecific
antibody



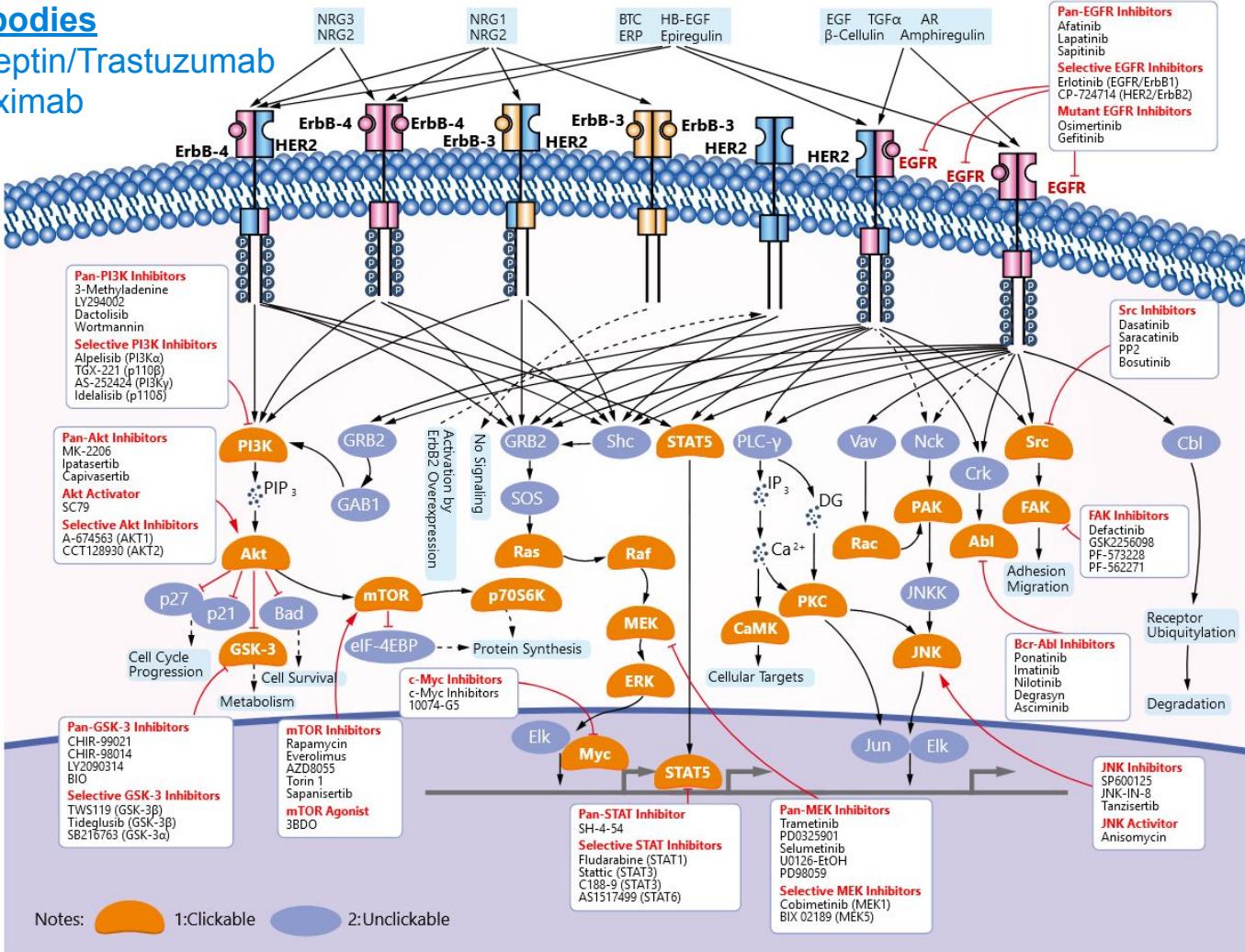
Chimeric
Antigen
Receptor
(CAR) T-cells

Multiple modalities can target the same biological process

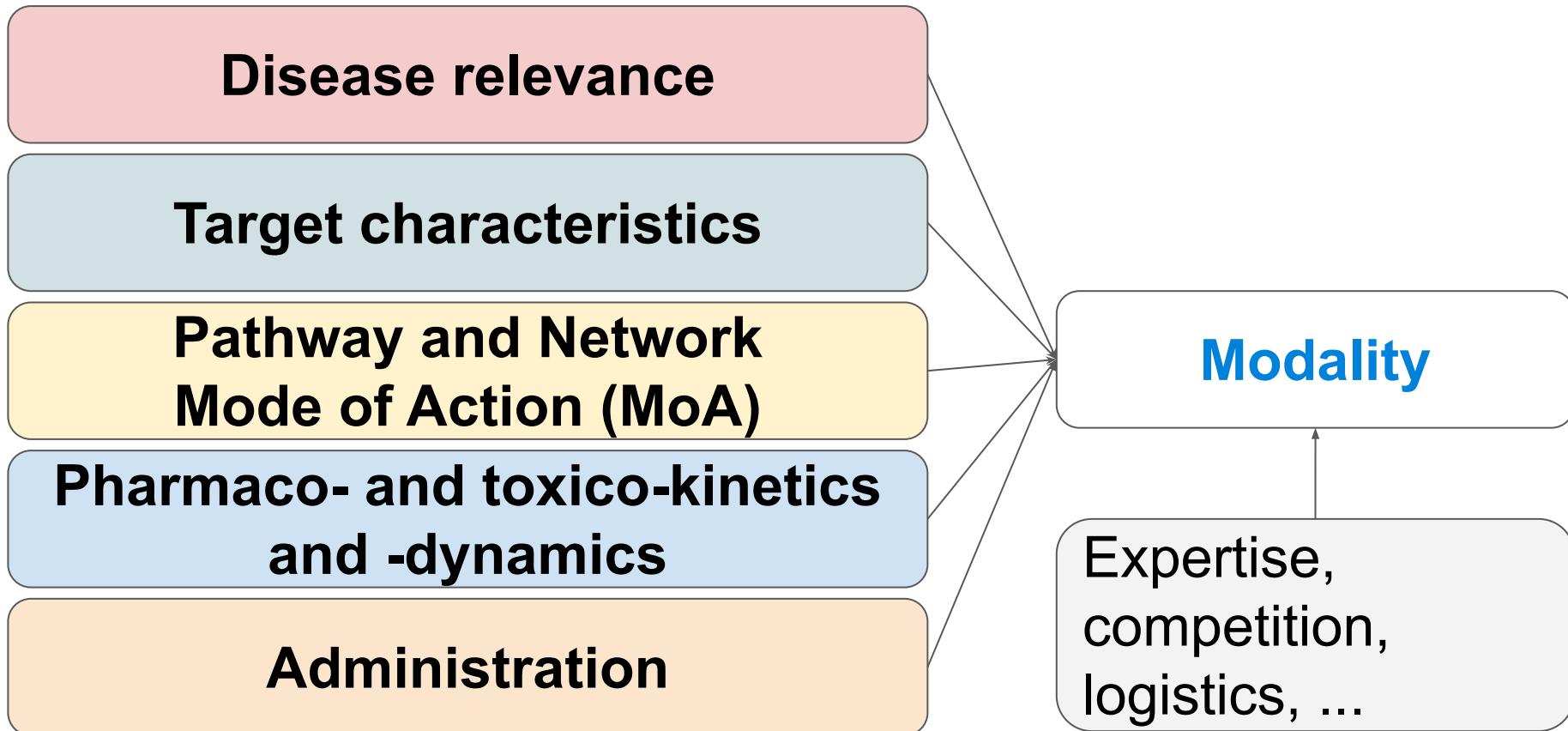
An example: the epidermal growth factor receptor (EGFR) pathway

Antibodies

Herceptin/Trastuzumab
Cetuximab



Criteria to choose a modality

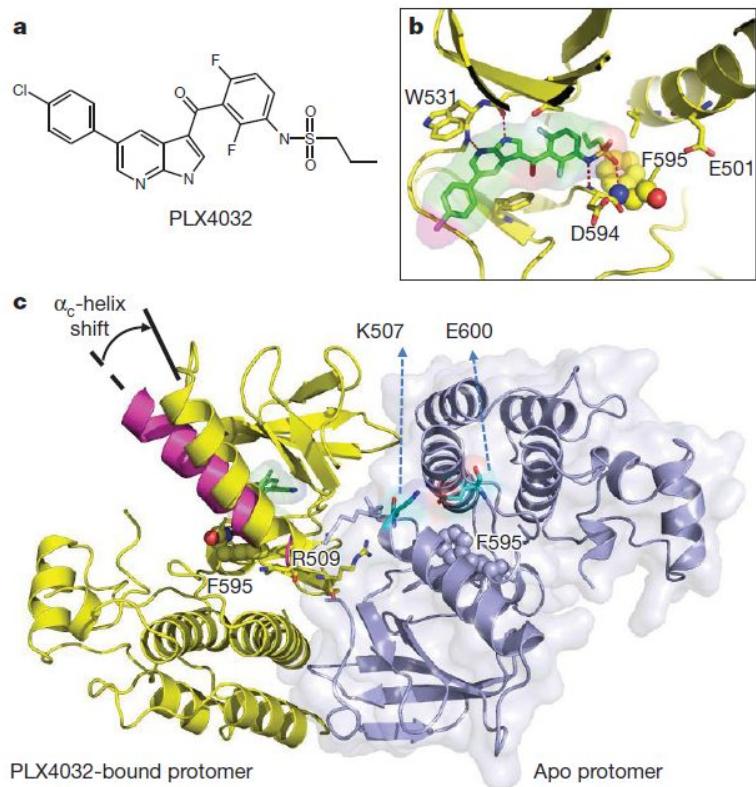


Characteristics of therapeutic modalities

Modality	Cause of disease at the protein level		Molecular target	Protein target localization			Delivery
	Reduction or loss of function	Excessive or detrimental function		Extracellular	Plasma membrane	Intracellular	
Small molecule	●	●	DNA → RNA → Protein	●	●	●	Oral Injection Inhaled
Protein replacement	○			○	○	○	○
Antibody		●		●	●		●
Oligonucleotide therapy	○	○	○	○	○	○	○
Cell and gene therapy*	●		●	●	●		●

Classical small molecules: an example from AMIDD

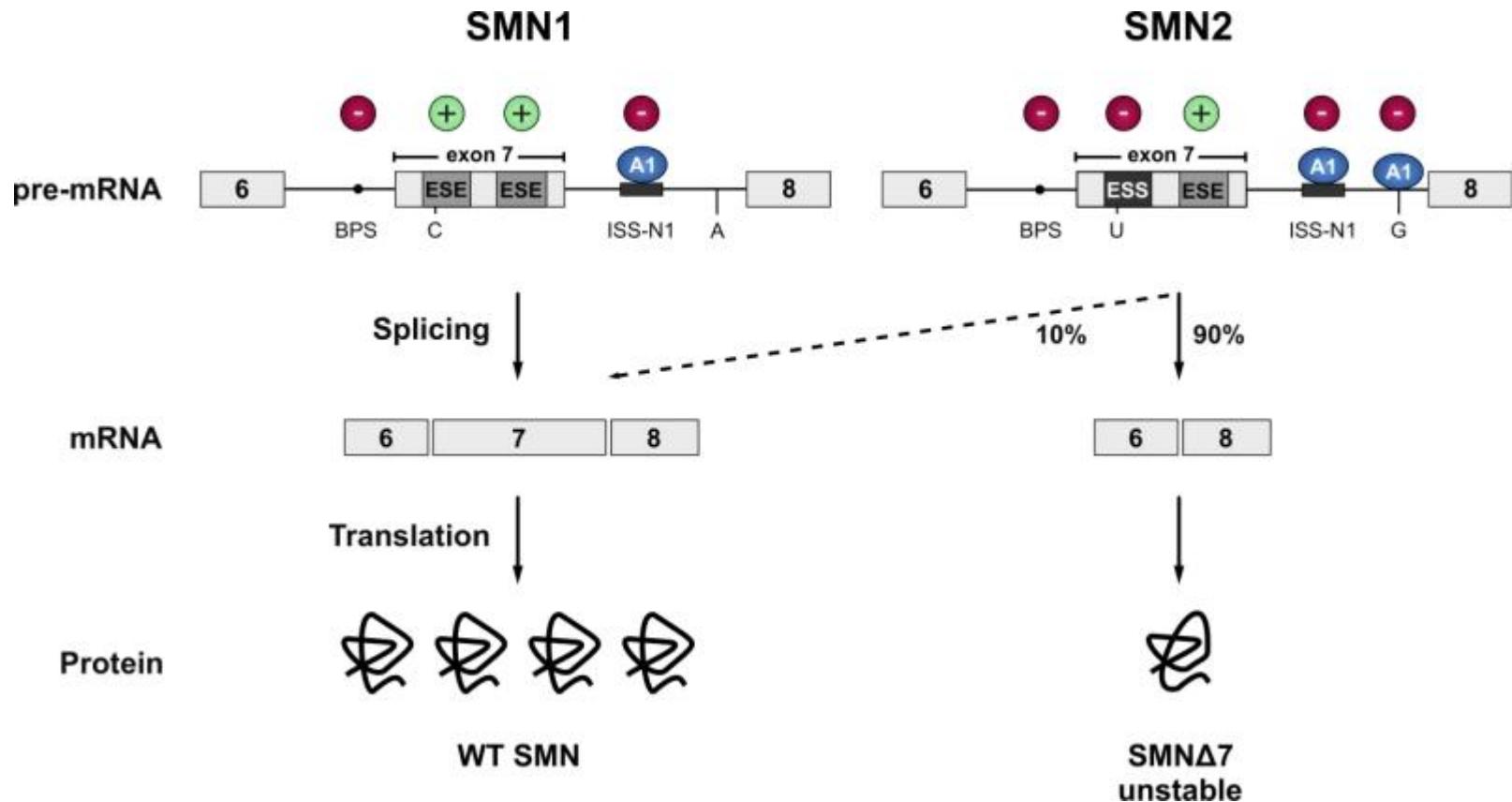
- Vemurafenib (Zelboraf, PLX4032)
V600E mutated BRAF inhibition
- Lock and key: an oversimplified yet powerful metaphor, first proposed by Emil Fischer



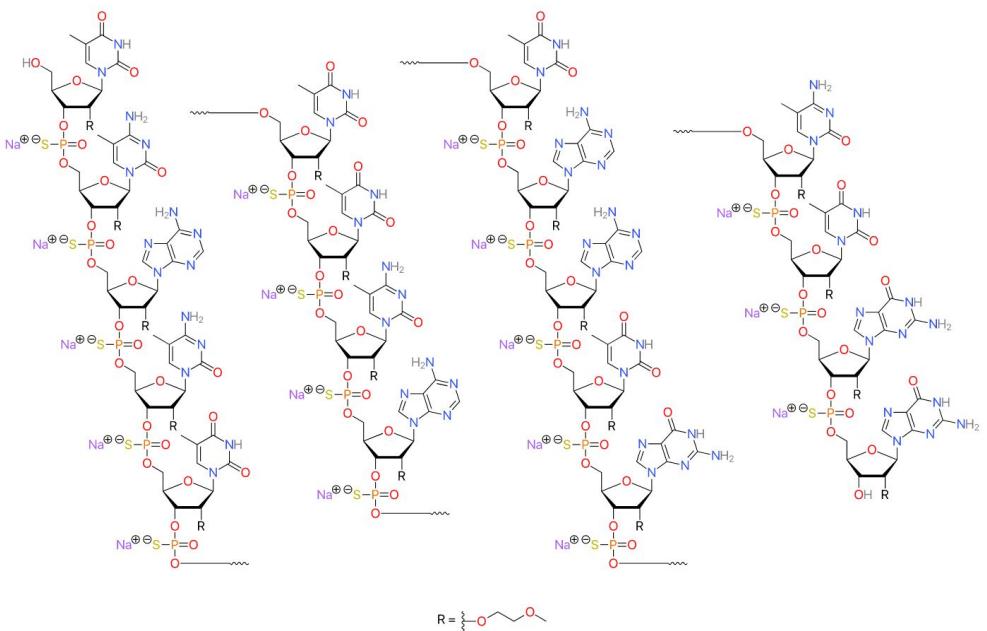
Facts about Spinal Muscular Atrophy (SMA)

- SMA is caused by a defect in a gene called *SMN1*. People with SMA have reduced levels of the SMN protein.
- When SMN protein levels are reduced, motor neurons are unable to send signals to the muscles, causing them to become smaller and weaker over time.
- Depending on the severity, or type of SMA, people with the disease will have difficulties moving, eating, and in some cases breathing, making them increasingly dependent on parents and caregivers.
- A short movie: <https://www.nejm.org/doi/full/10.1056/NEJMoa2009965>

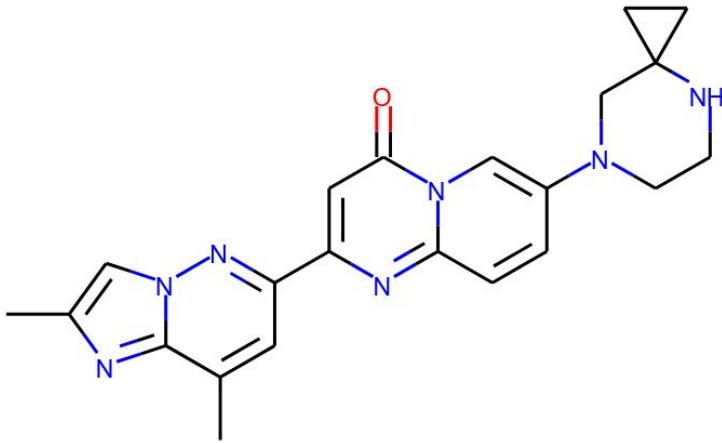
The molecular mechanism of SMA



Two Drugs, One Disease

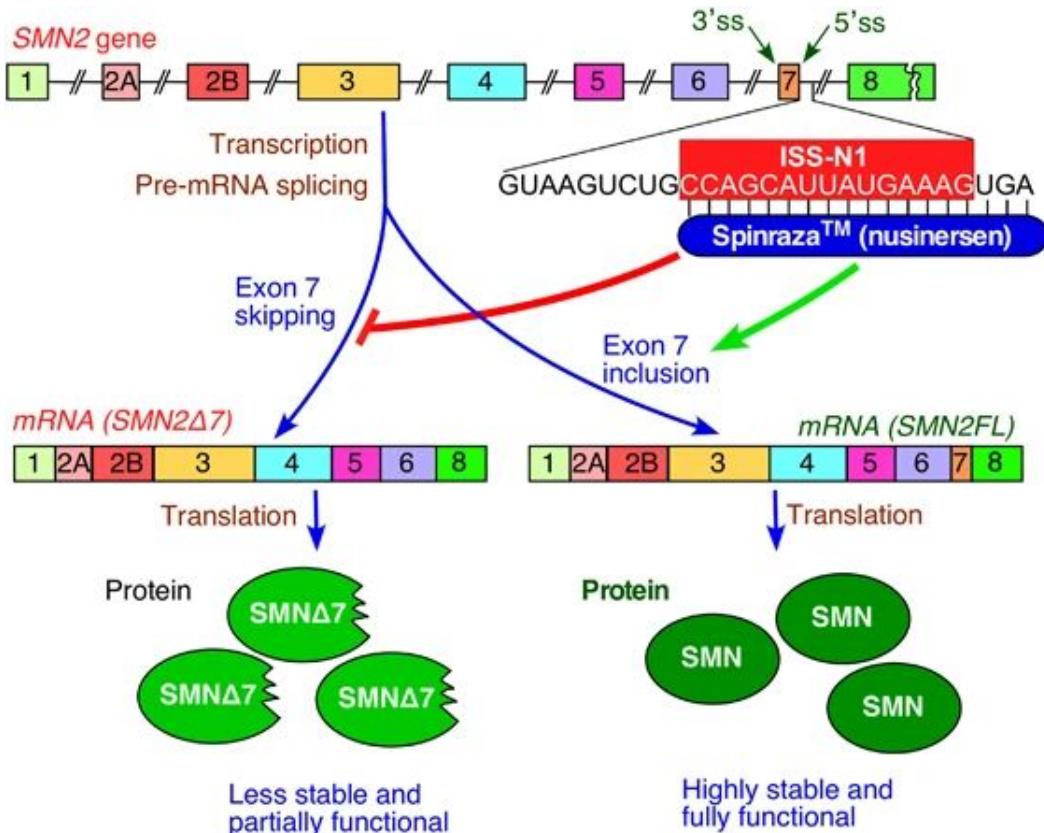


Nusinersen sodium/ Spinraza
[\(CHEMBL3833342\)](#)

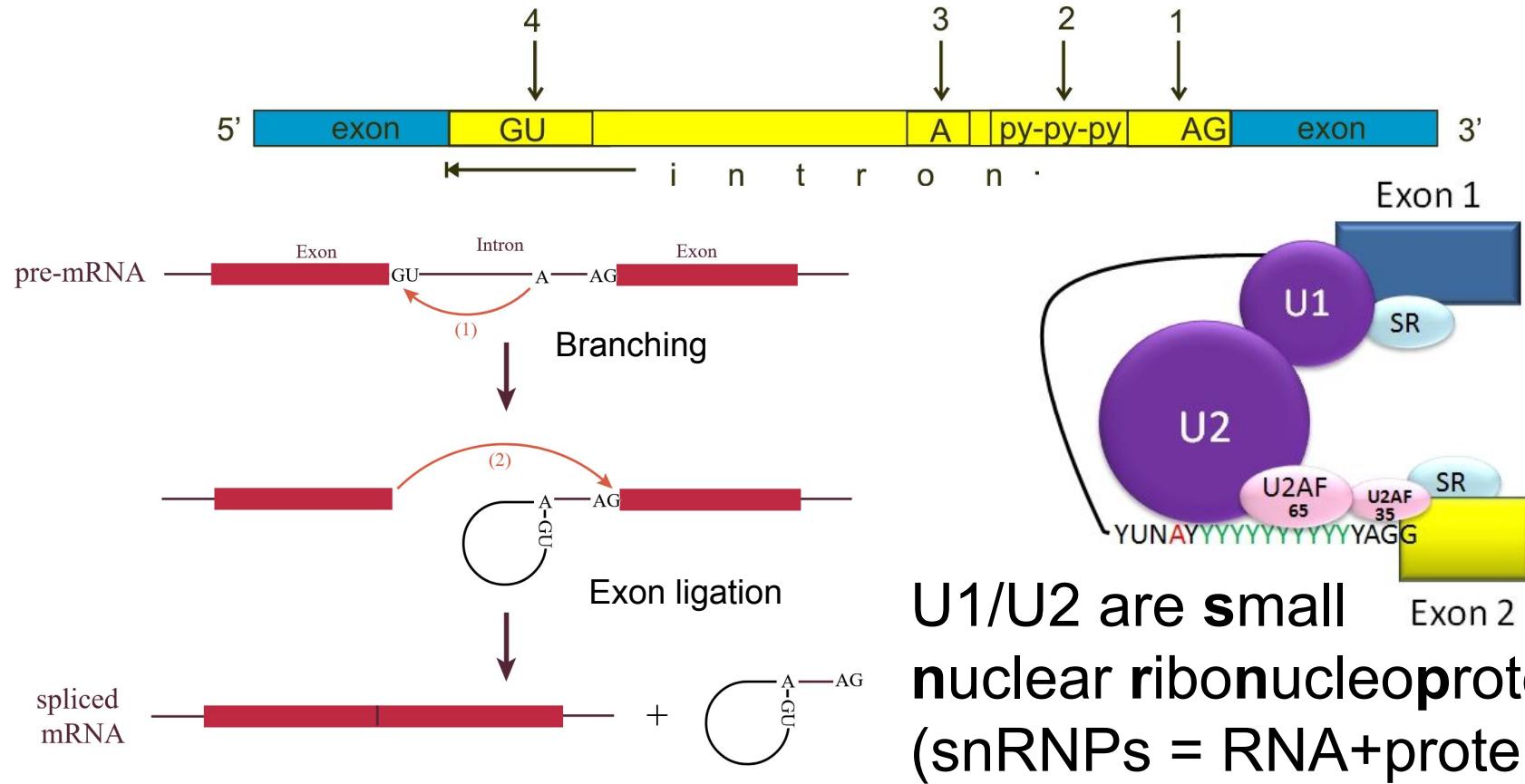


Risdiplam/ Evrysdi/
[\(CHEMBL4297528\)](#)

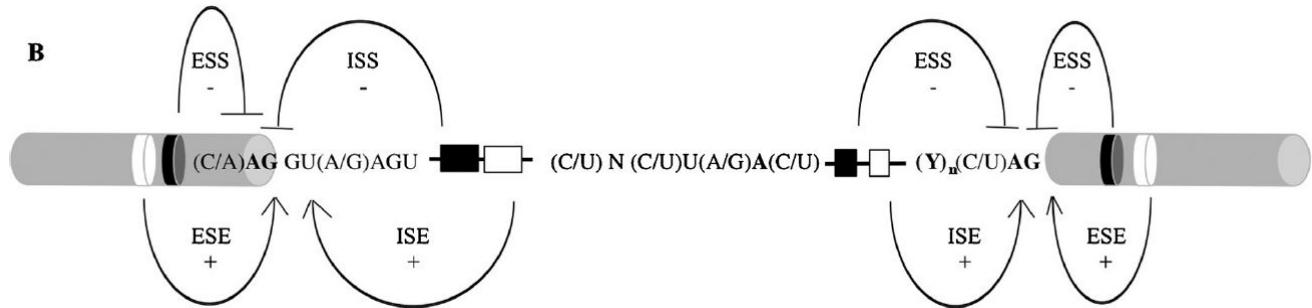
How Spinraza (nusinersen) works



Spliceosome: the splicing machinery



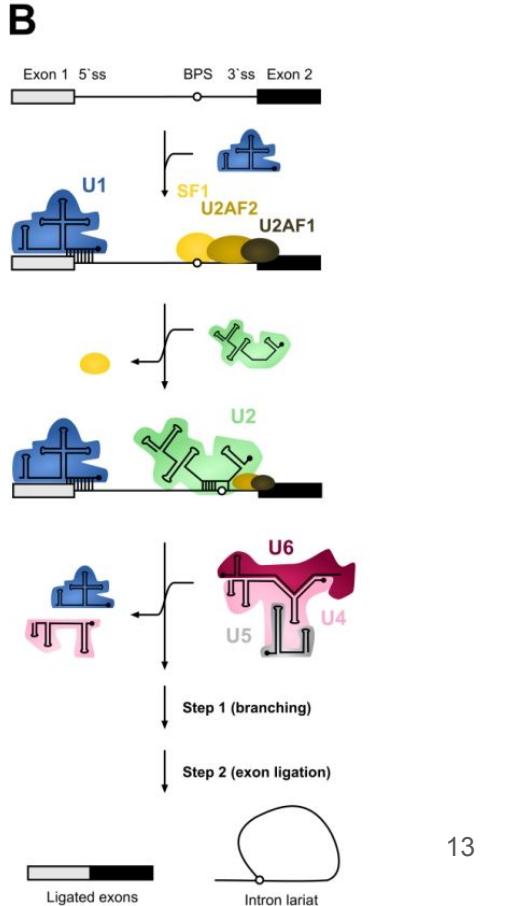
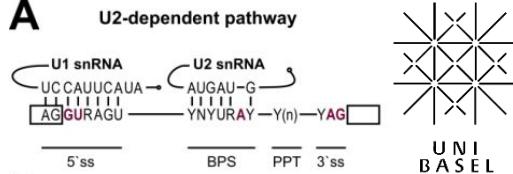
Splicing in action and under regulation



ESS=exon splicing silencer; ESE=exon splicing enhancer;

ISS=intron splicing silencer; ISE=intron splicing enhancer.

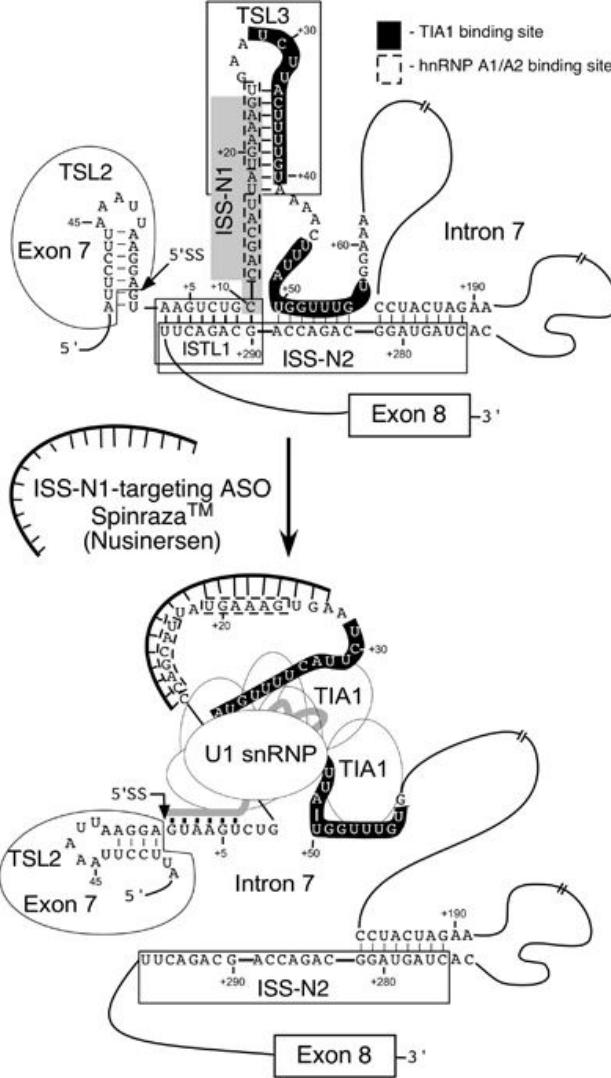
BPS=branch point sequence; PPT=polypyrimidine tract (C/U);



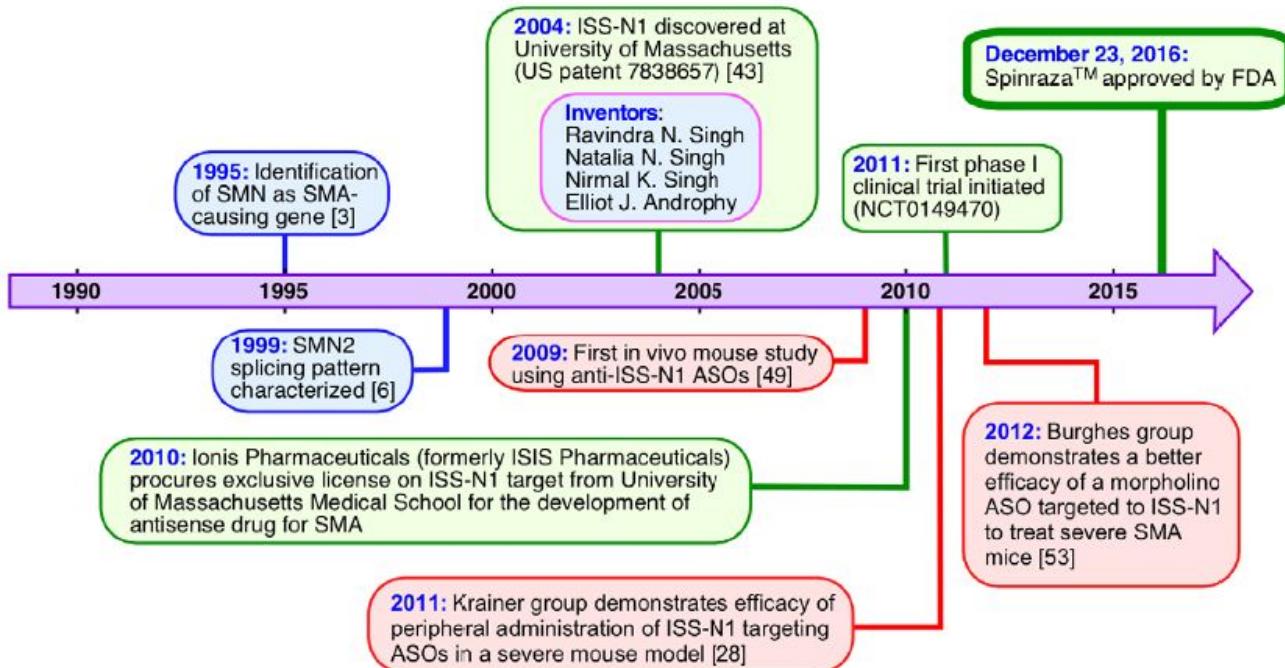
How Spinaraza (nusinersen) works, base by base

Nusinersen binds to ISS-N1, causing structural rearrangement and recruitment of U1 snRNP by TIA1.

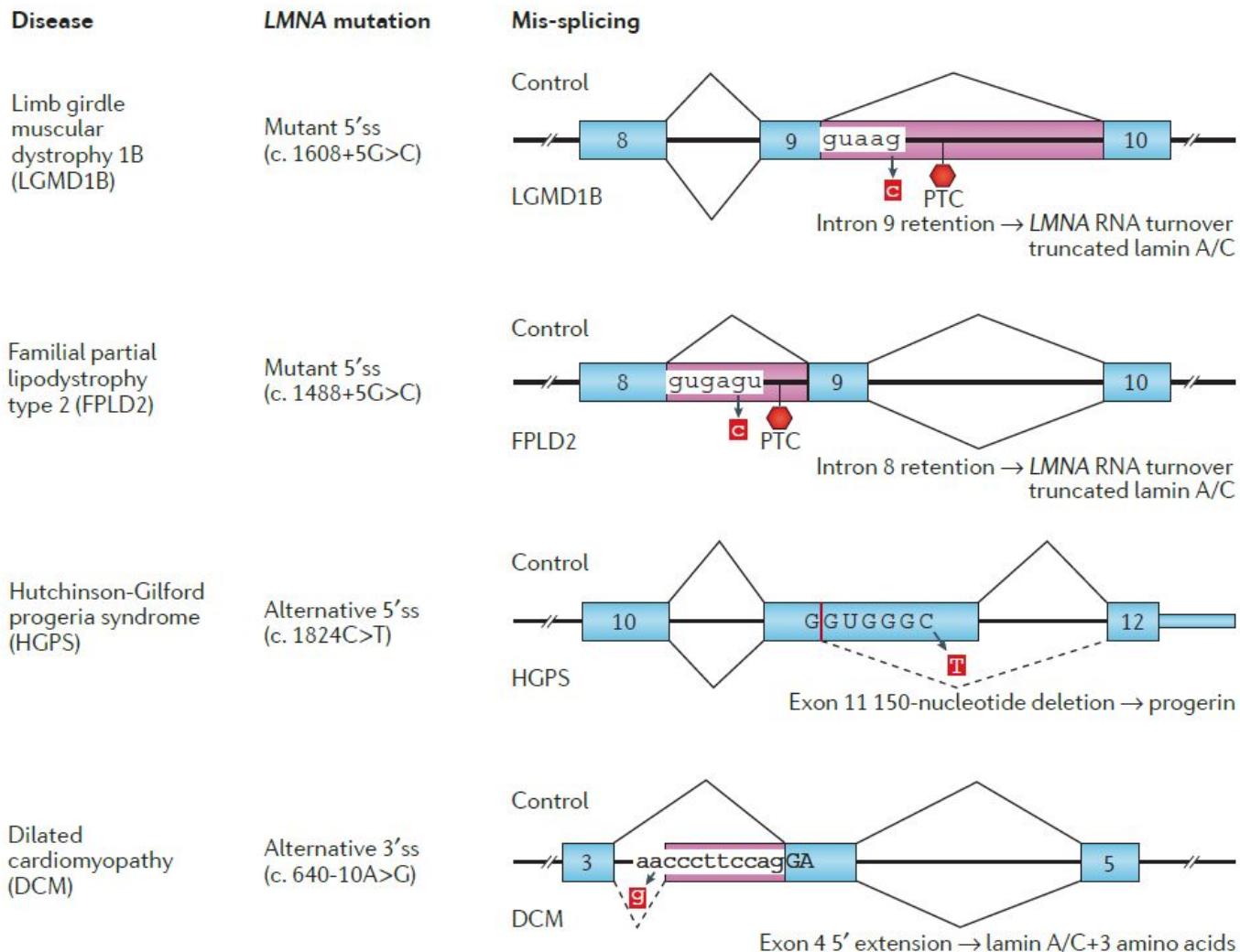
- ISS-N1: Intronic splicing silencer N1;
- TIA1: TIA1 cytotoxic granule associated RNA binding protein;
- TSLs: (inhibitory) terminal stem-loop structures;
- ISTL1: internal stem formed by a long-distance interaction



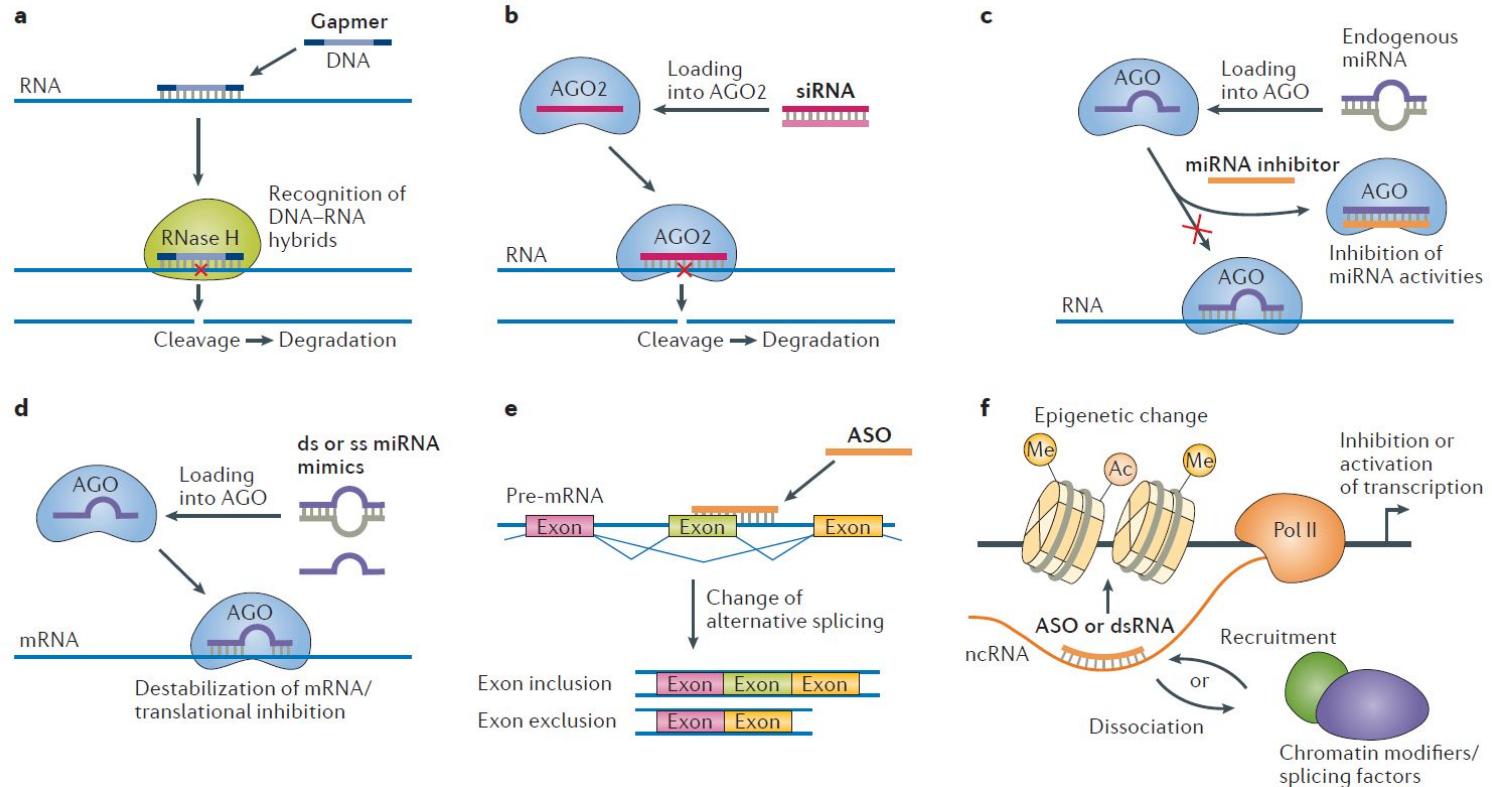
It takes 21 years to go from a molecular model to a population model



Splicing modifying oligo-nucleotide and other RNA therapeutics have strong disease relevances

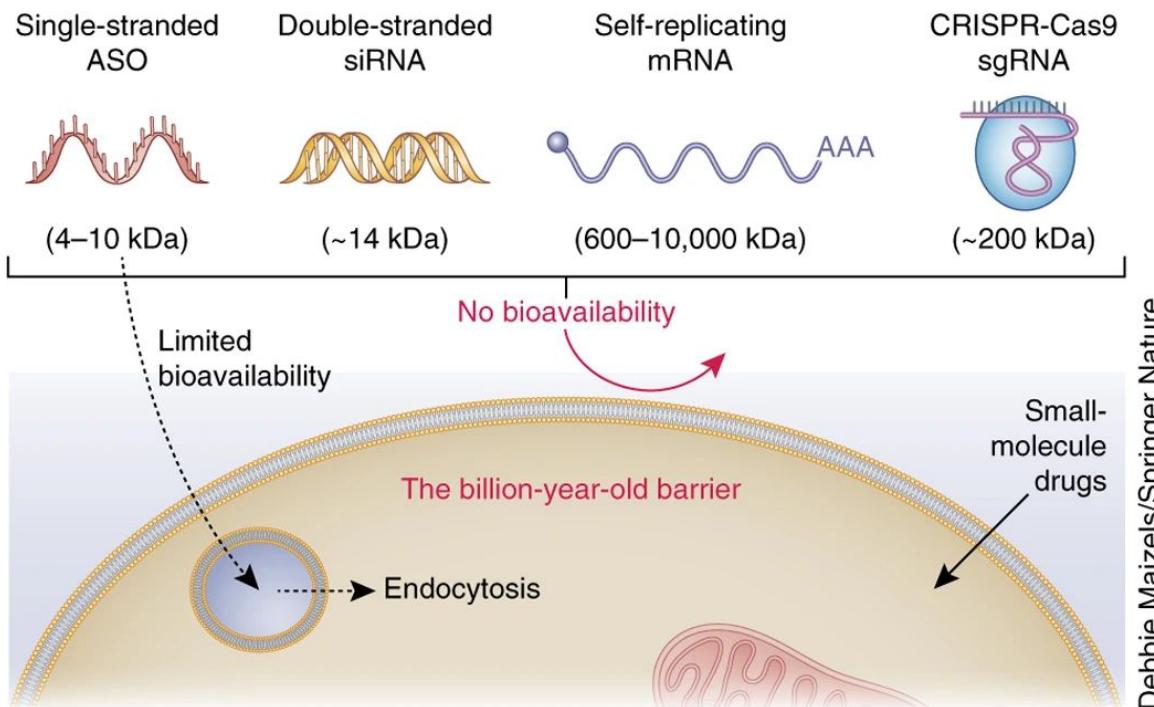


Regulating RNA levels or splicing with ASOs and duplex RNAs



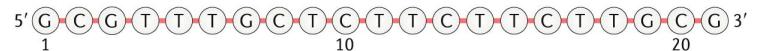
The four-billion-year-old barrier to RNA therapeutic

- Too large and charged to pass lipid bilayers
- Degradable by RNases
- Rapid clearance from liver and kidney
- Immunogenicity
- Endocytosis
- Delivery into organs other than liver and eye

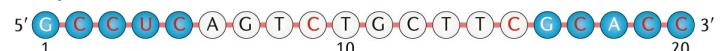


Chemistry of oligonucleotides evolves with time

a Fomivirsen



b Mipomersen



c Inotersen



d Eteplirsen



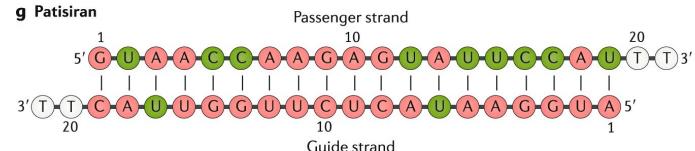
e Golodirsen



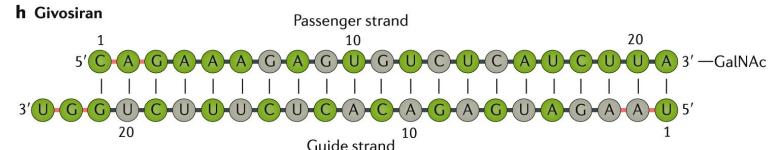
f Nusinersen



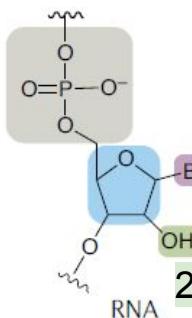
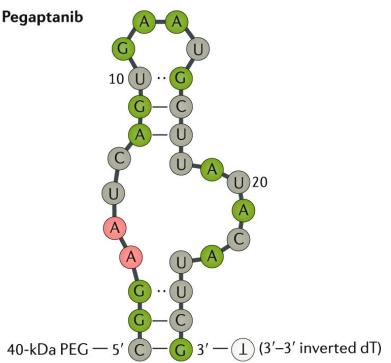
g Patisiran



h Givosiran



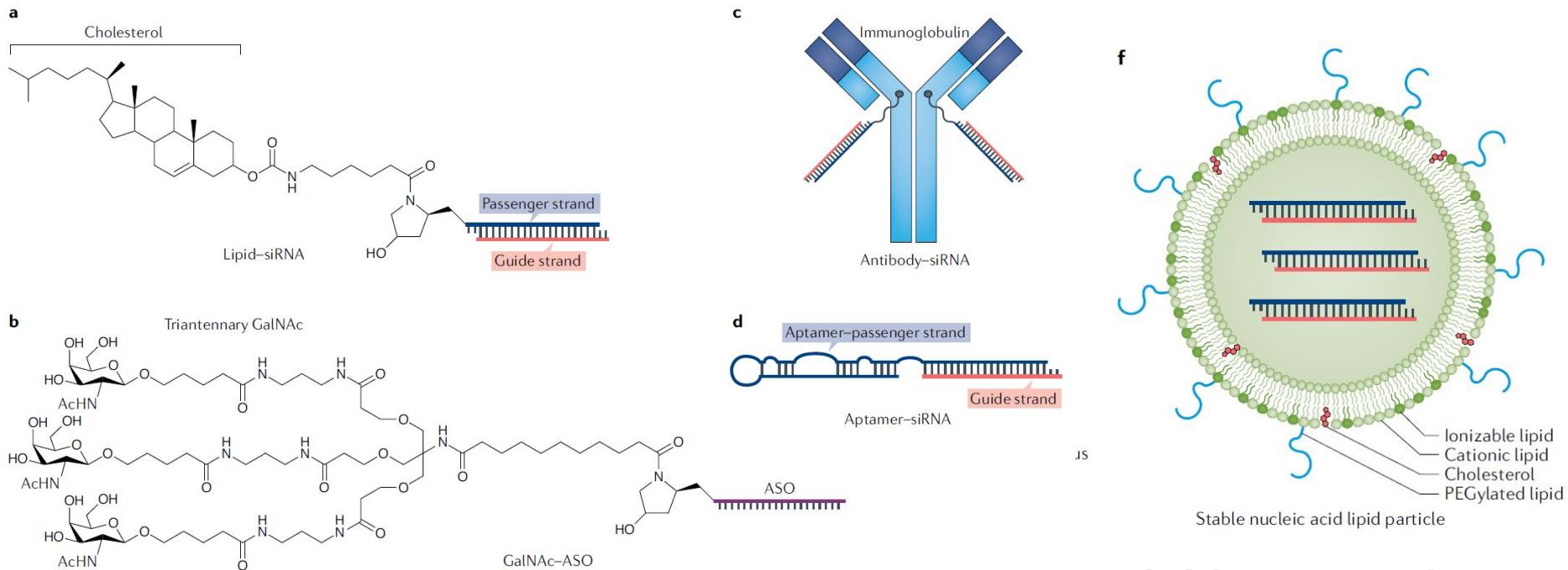
i Pegaptanib



(N)	DNA
N	RNA
N	PMO
N	2'-O-methoxyethyl
N	2'-O-methyl
N	2'-Fluoro
Y	5-Methyl pyrimidine
—	Phosphorothioate
—	Phosphodiester

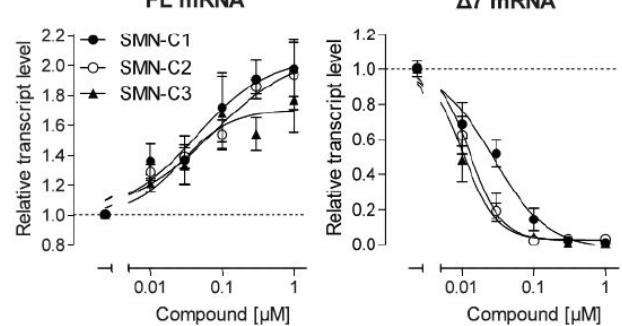
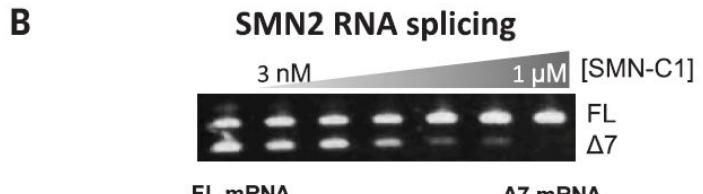
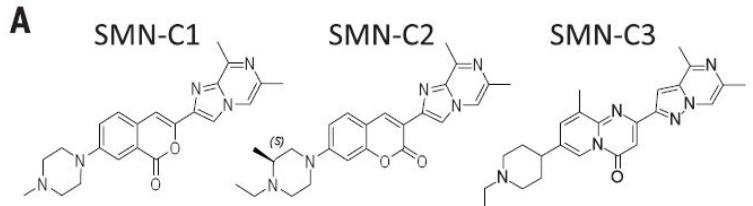
PMO=phosphorodiamidate morpholino oligomer

Delivery systems of antisense oligonucleotides



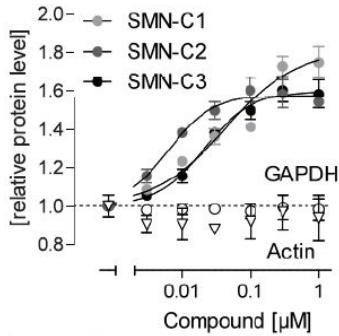
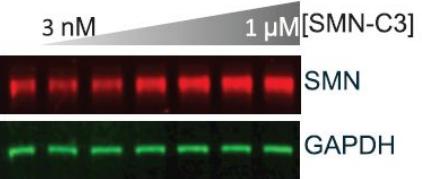
lipid nanoparticles

Small molecules as RNA splicing modifiers



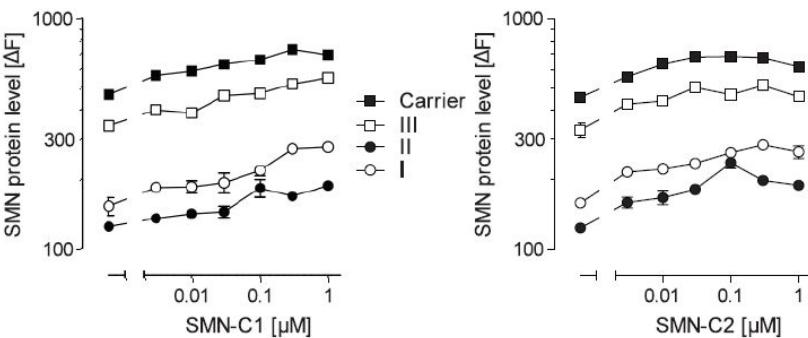
C

SMN protein (Western Blot)



D

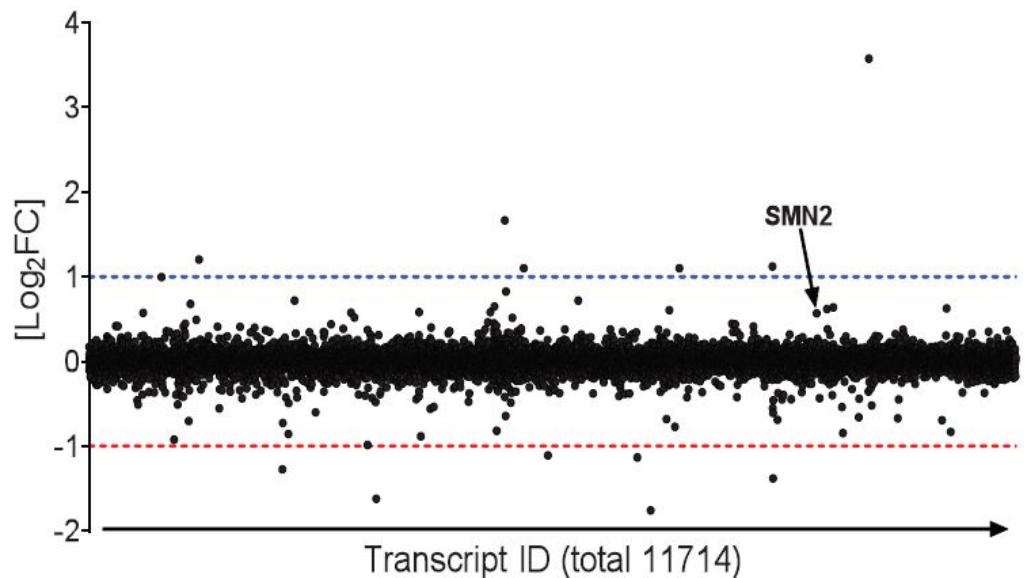
SMN protein (HTRF)



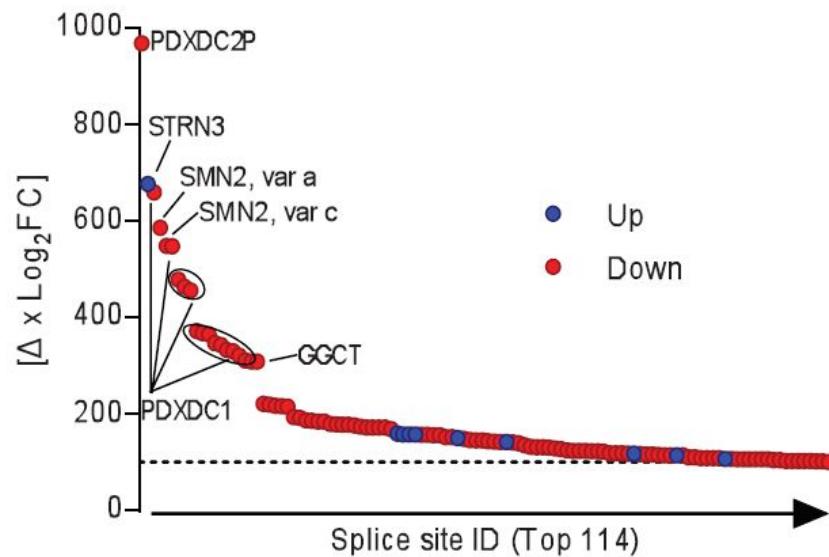
RNA sequencing confirms the specificity of SMN-C3

A

Transcriptional changes by SMN-C3

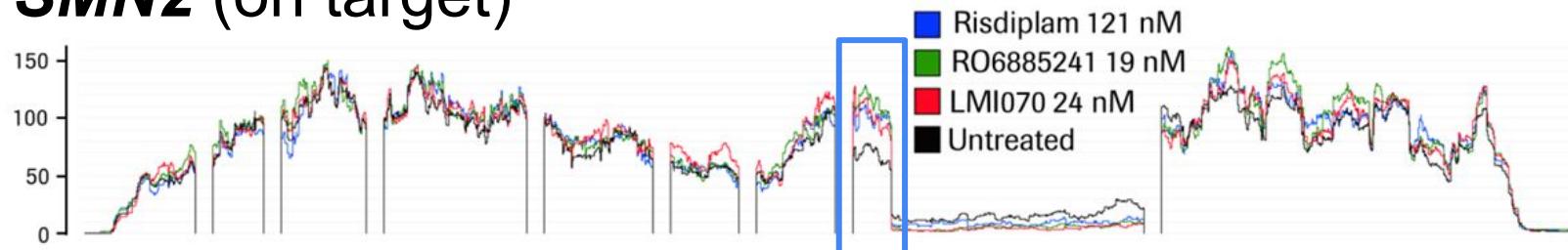

B

Splicing regulation by SMN-C3

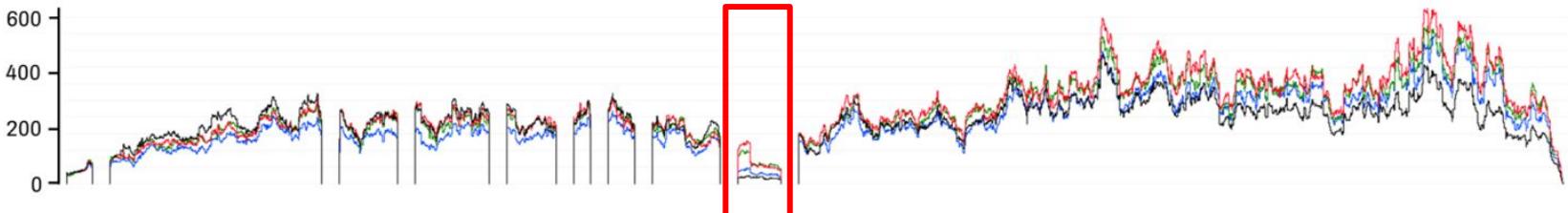


RNA sequencing confirms the specificity of SMN-C3

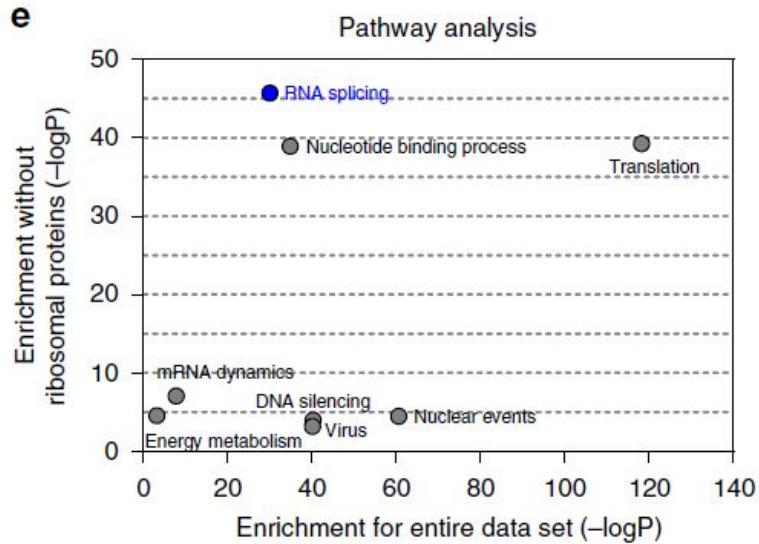
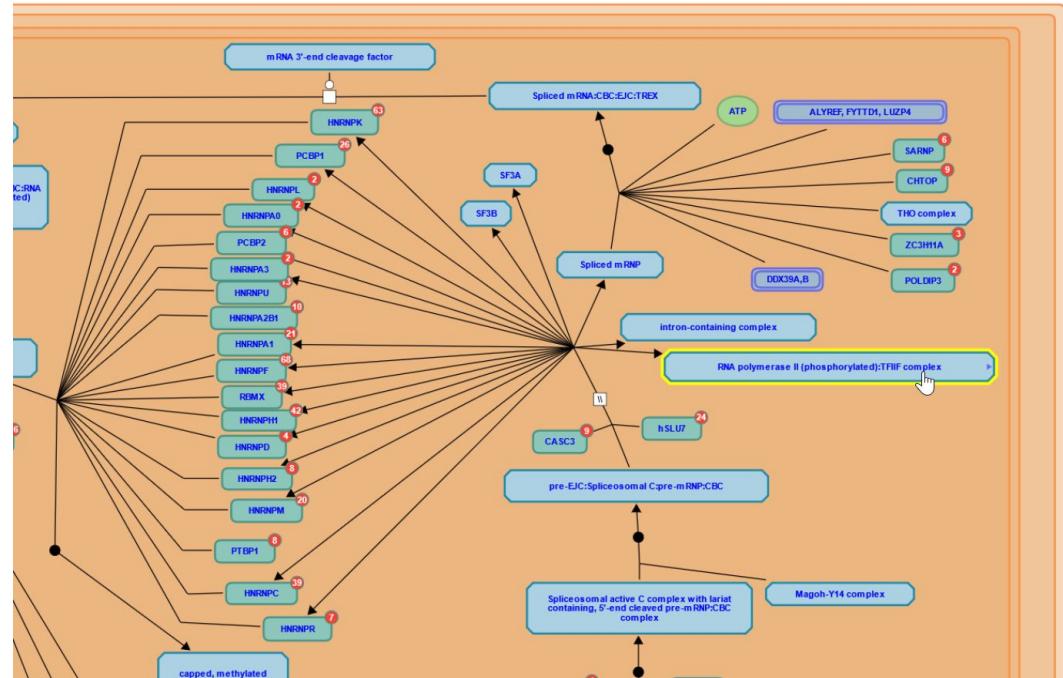
SMN2 (on target)



FOXM2 (off target)

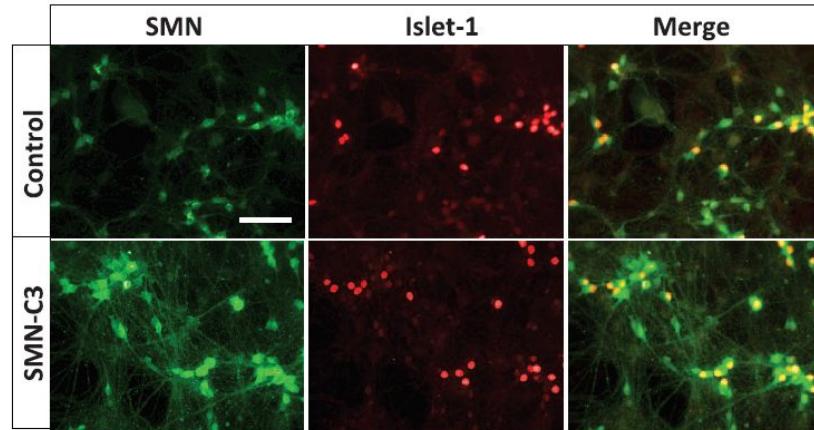


Gene-enrichment analysis confirms specific regulation of RNA splicing



Part of the mRNA splicing pathway in Reacome

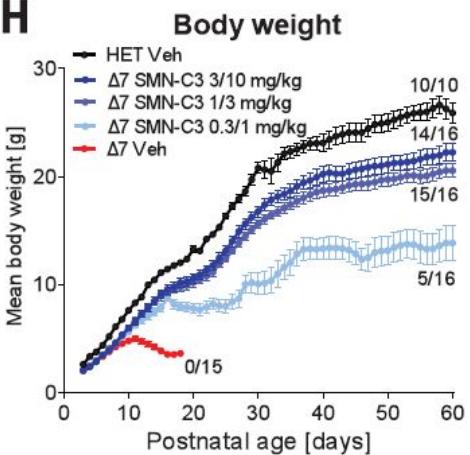
Experiments *in vitro* and *in vivo* support efficacy profiles of SMN-C3



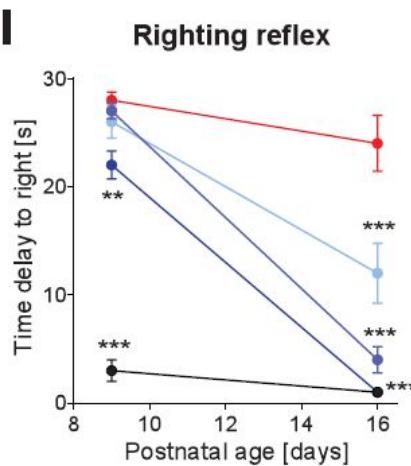
G



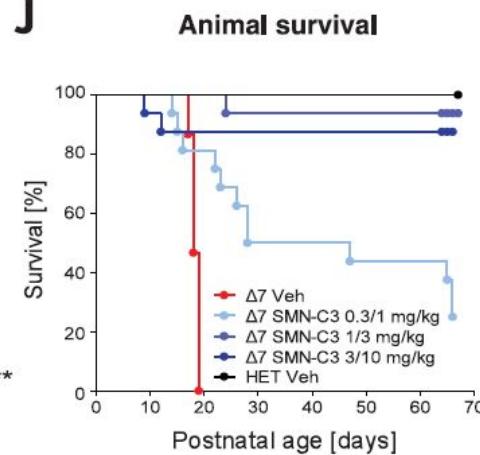
H



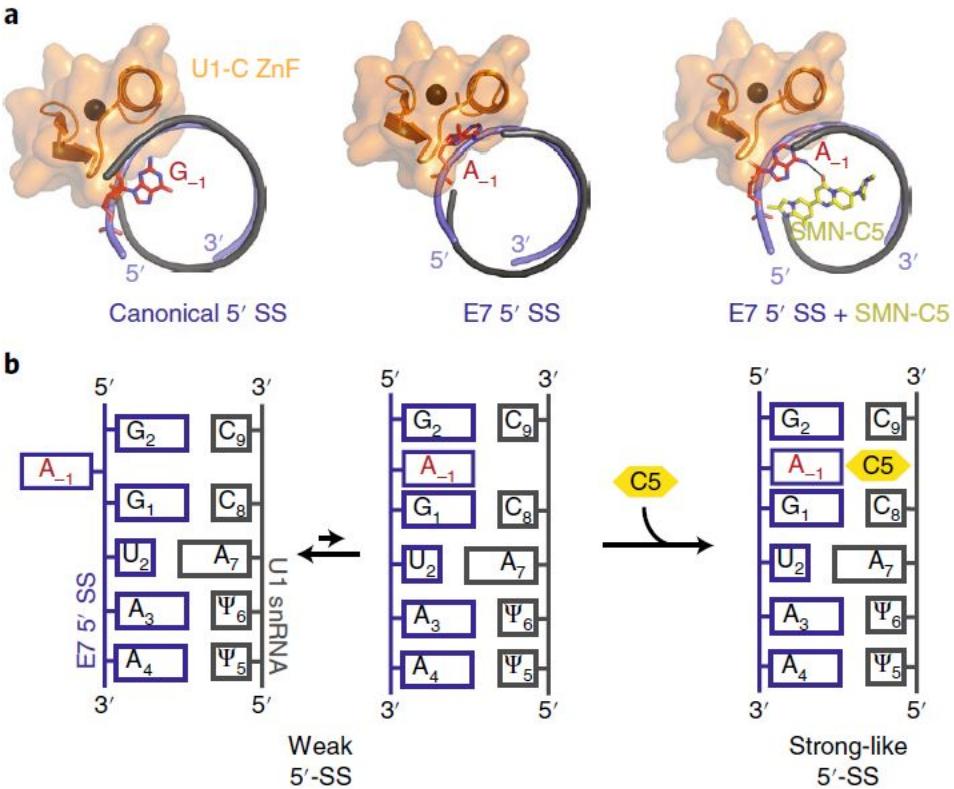
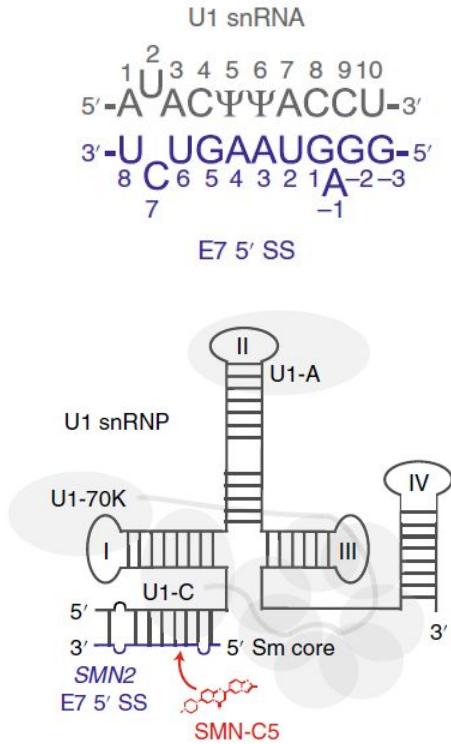
I

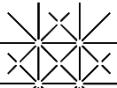


J



Structural basis of specific splicing correction





Clinical trial (FIREFISH Part 1) Results

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.*

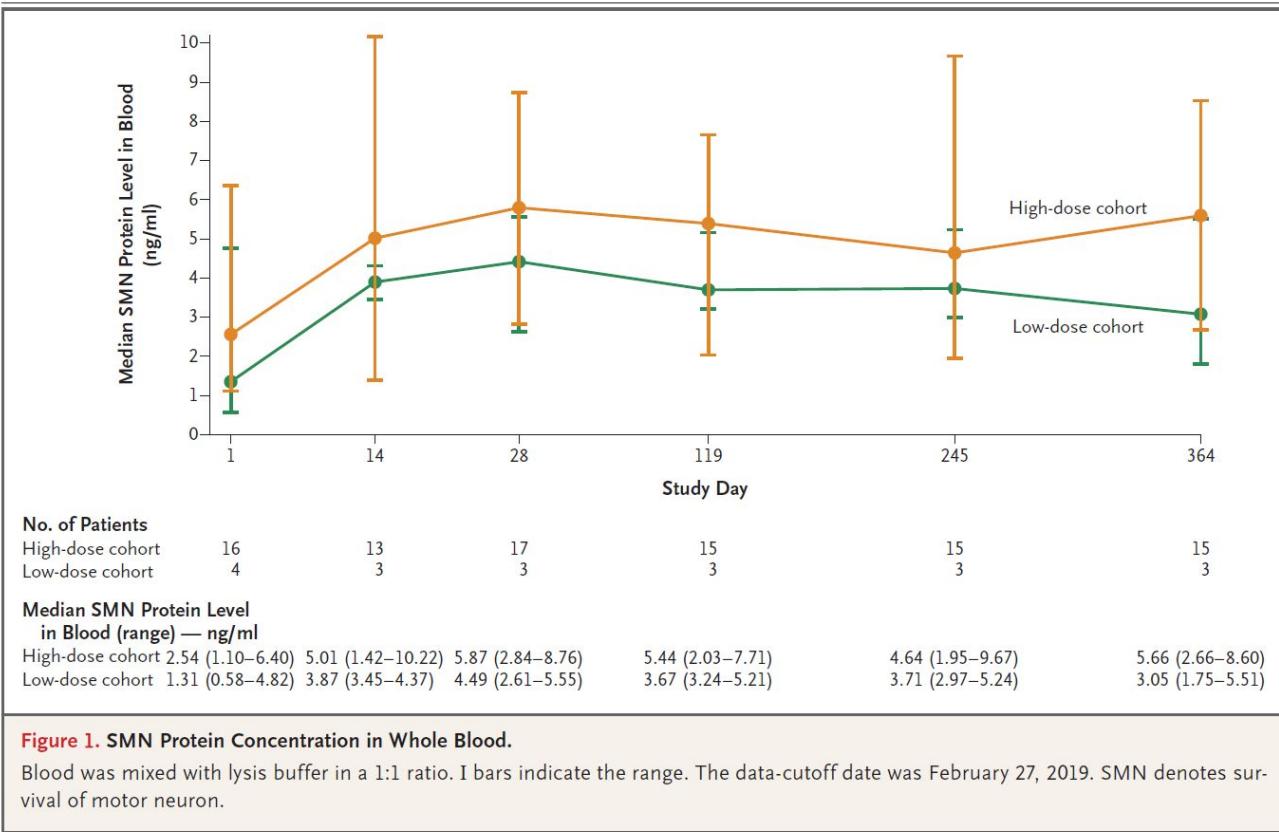
Characteristic	Low-Dose Cohort (N=4)	High-Dose Cohort (N=17)	All Infants (N=21)
Sex — no. (%)			
Female	4 (100)	11 (65)	15 (71)
Male	0	6 (35)	6 (29)
Median age (range) — mo			
At onset of symptoms	2.7 (2.0–3.0)	1.5 (0.9–3.0)	2.0 (0.9–3.0)
At diagnosis	3.3 (2.5–5.1)	3.0 (0.9–5.4)	3.0 (0.9–5.4)
At enrollment	6.9 (6.7–6.9)	6.3 (3.3–6.9)	6.7 (3.3–6.9)
Motor measures†			
Median CHOP-INTEND score (range)	23.5 (10–25)	24 (16–34)	24 (10–34)
Median HINE-2 score (range)	1 (0–3)	1 (0–2)	1 (0–3)
Respiratory support — no. (%)	0	5 (29)‡	5 (24)‡

Note: Table 2 is not complete

Table 2. Adverse Events.*

Event	Infants (N=21)
Total no. of adverse events	202
≥1 Adverse event — no. (%)	21 (100)
Total no. of serious adverse events	24
≥1 Serious adverse event — no. (%)	10 (48)
≥1 Adverse event of grade 3–5 — no. (%)	9 (43)
Serious adverse event with fatal outcome — no. (%)†	3 (14)
Most common adverse events — no. (%)‡	
Pyrexia	11 (52)
Upper respiratory tract infection	9 (43)
Diarrhea	6 (29)
Cough	5 (24)

Clinical trial (FIREFISH Part 1) Results



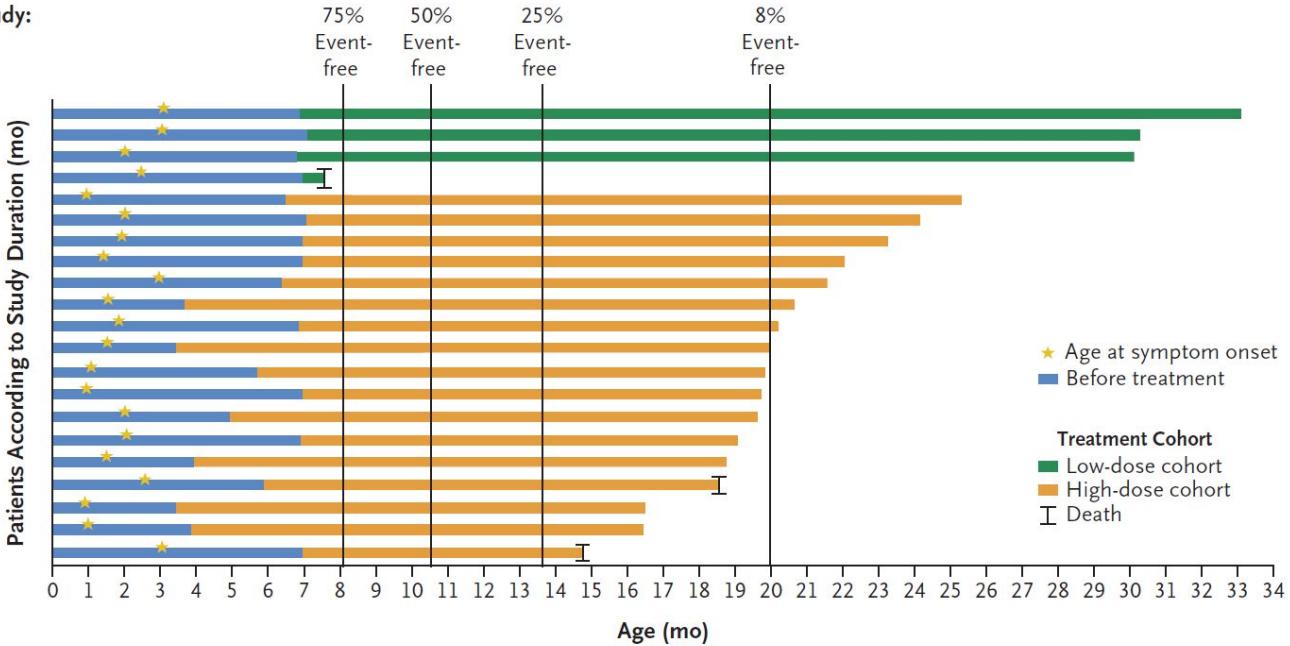
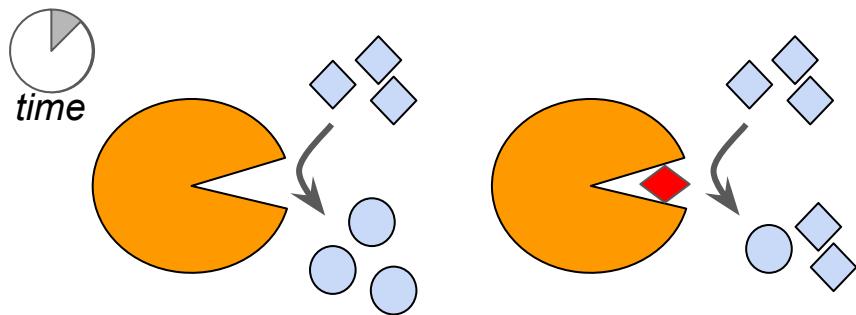
Natural History Study:


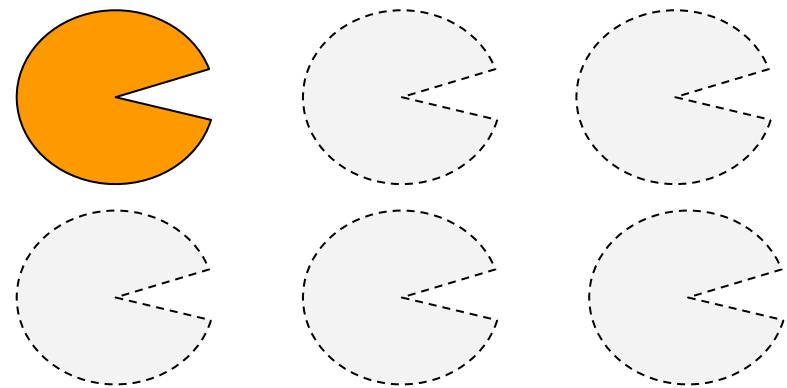
Figure 2. Event-free Survival.

Event-free survival was defined as being alive and not receiving permanent ventilation (tracheostomy or ventilation [bilevel positive airway pressure] for ≥ 16 hours per day continuously for >3 weeks or continuous intubation for >3 weeks, in the absence of, or after the resolution of, an acute reversible event). The percentages of patients who were event-free in a previous natural history study of spinal muscular atrophy⁷ are shown at the top of the graph for comparison. The median age at the combined outcome among patients in the previous study who had two copies of *SMN2* was 10.5 months (interquartile range, 8.1 to 13.6); event-free survival in that study was defined as being alive and not receiving noninvasive ventilation for 16 hours or more per day continuously for 2 or more weeks. The duration of our study was measured from the date of enrollment to the data-cutoff date. As of the data-cutoff date, three infants (one in the low-dose cohort and two in the high-dose cohort) had died; one additional infant in the high-dose cohort died after that date (Table S5).

Competitive inhibitors reduce reaction rate; antisense oligonucleotides modulate protein abundance



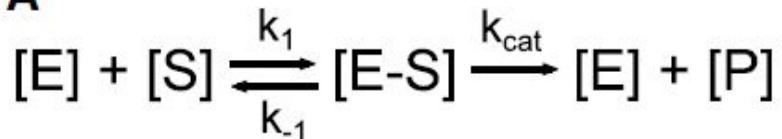
A competitive inhibitor (red diamond) reduces the rate of product generation in an enzymatic reaction.



Antisense oligonucleotides reduce the abundance of the enzyme protein.

Enzymic and genetic inhibition have distinct impact on reaction dynamics

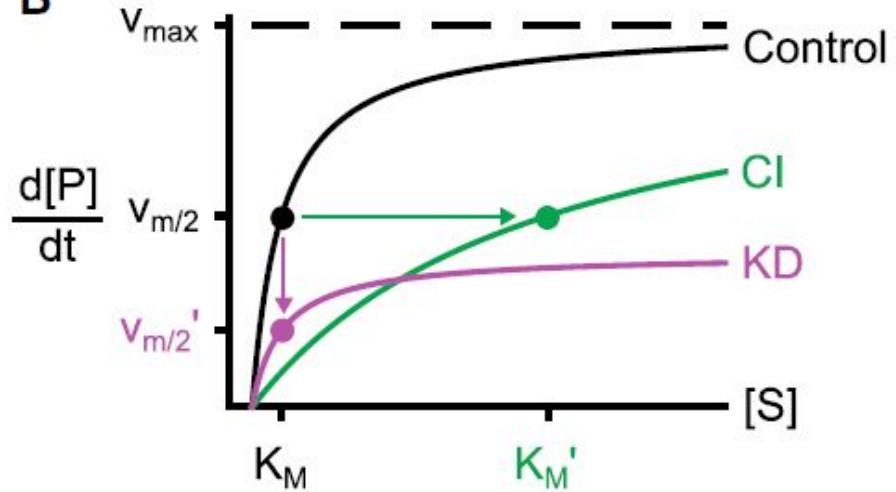
A



$$\frac{d[P]}{dt} = v_{\max} \frac{[S]}{[S] + K_M}$$

$$K_M = \frac{k_{-1} + k_{\text{cat}}}{k_1} \quad v_{\max} = k_{\text{cat}}[E]_o$$

B

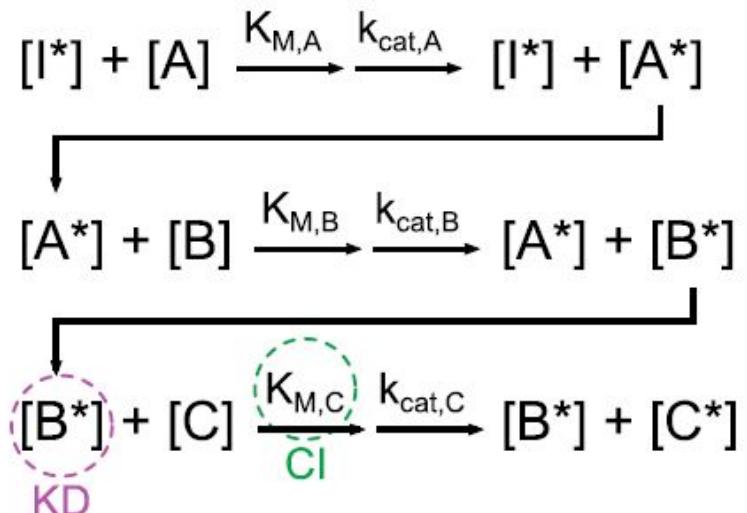


The Michaelis-Menten Equation

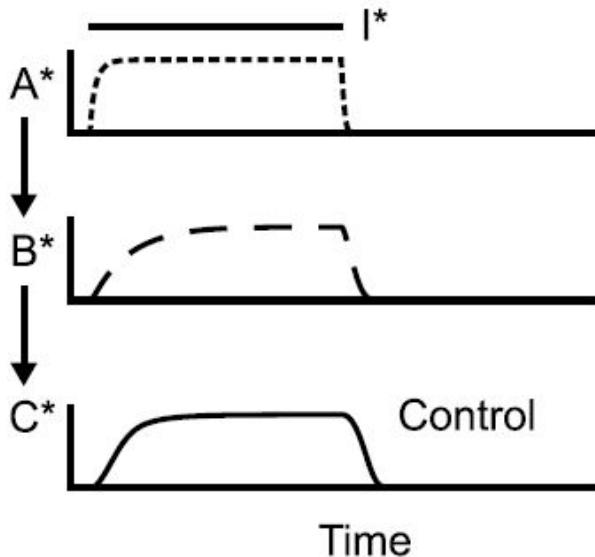
Competitive inhibition (CI)
versus knockdown (KD)

A linear system simulating enzymatic reactions

C



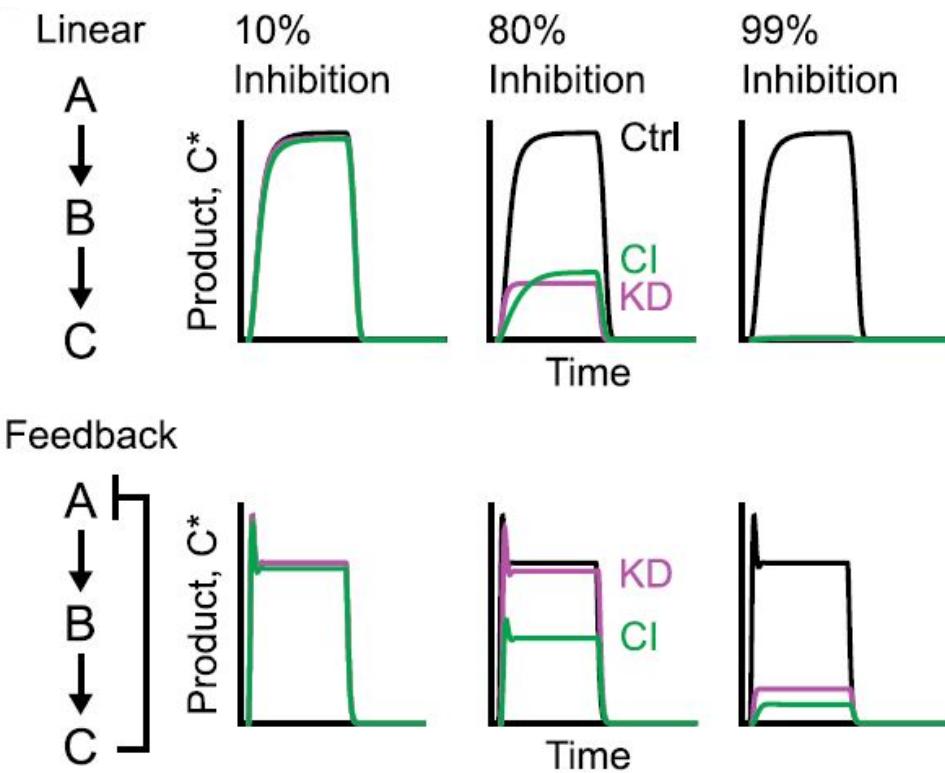
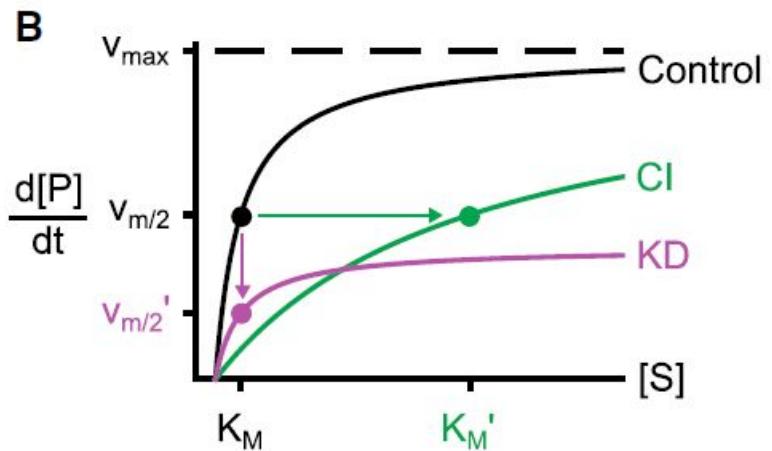
D



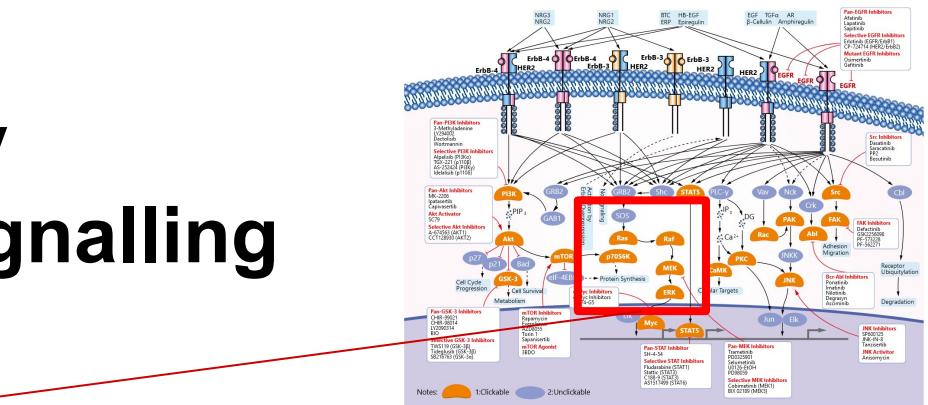
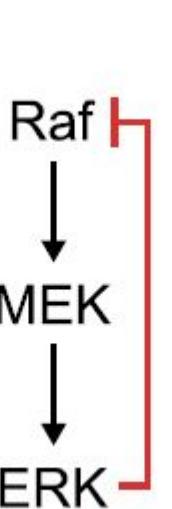
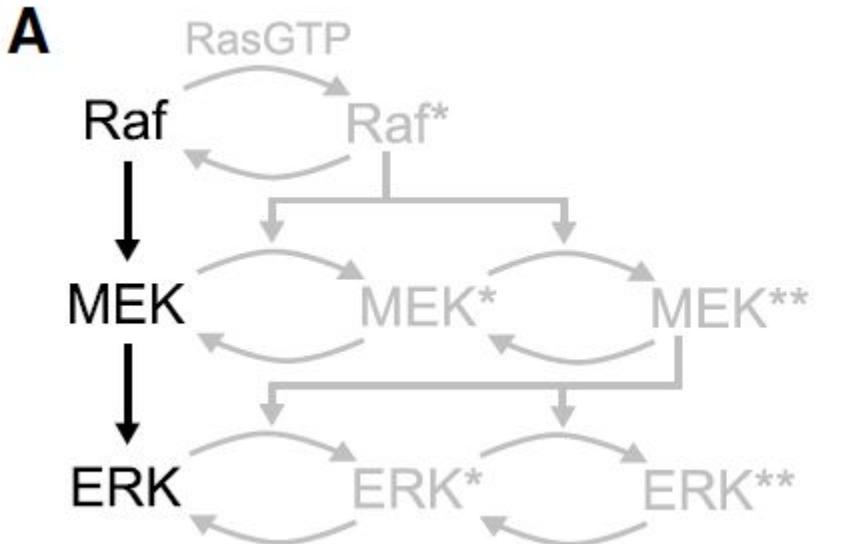
I^* : upstream input; A/A^* and B/B^* : inactivated and activated enzyme; C^* : product

Adding a negative feedback may differentiate effects of enzymatic and genetic inhibition

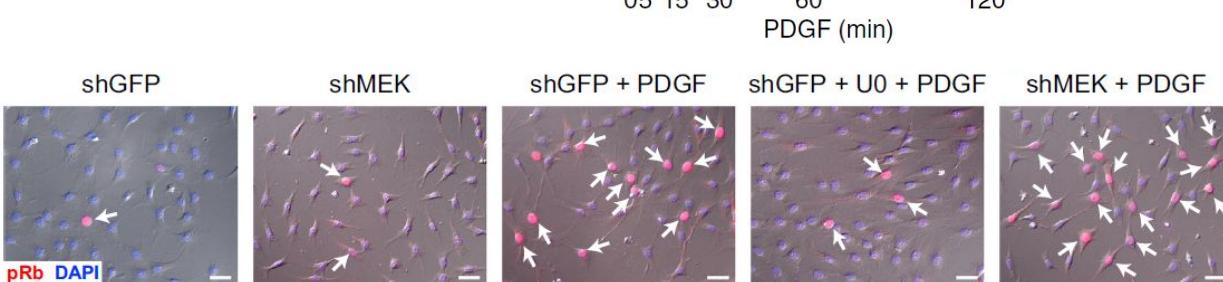
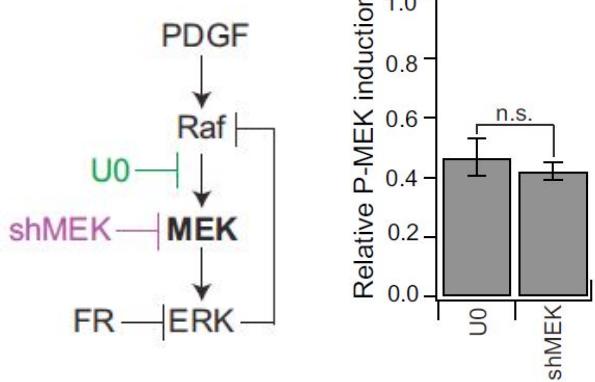
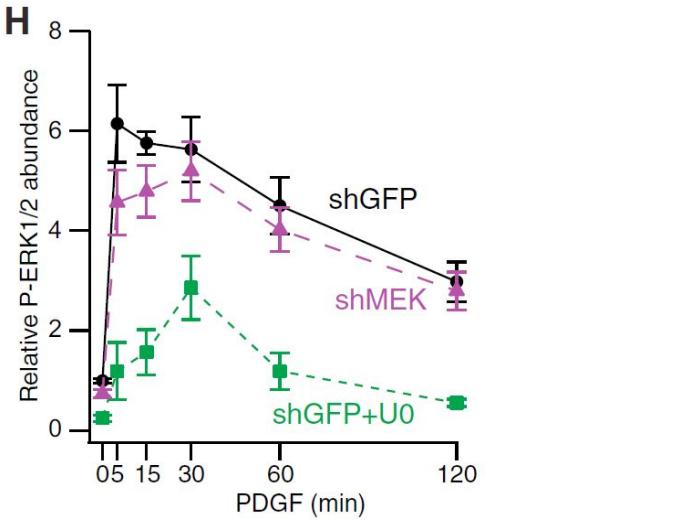
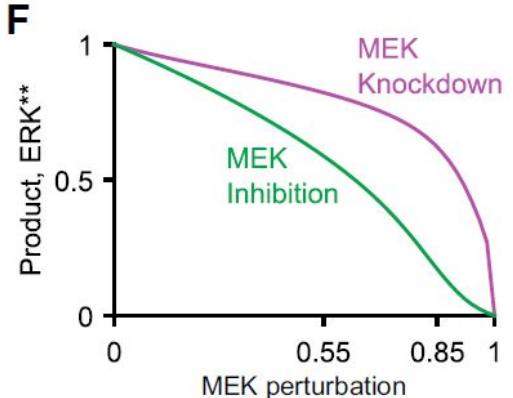
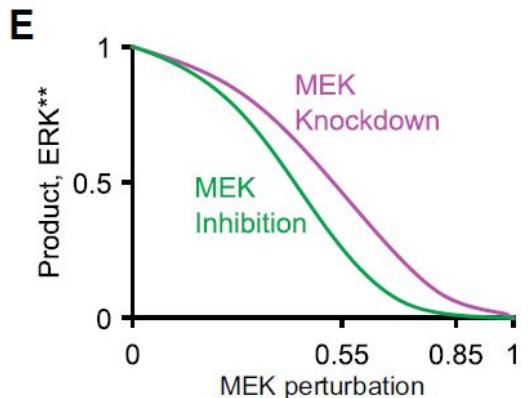
Intuition: when $[B^*]$ stays low, CI leads to **slower** accumulation of C^* than KD.



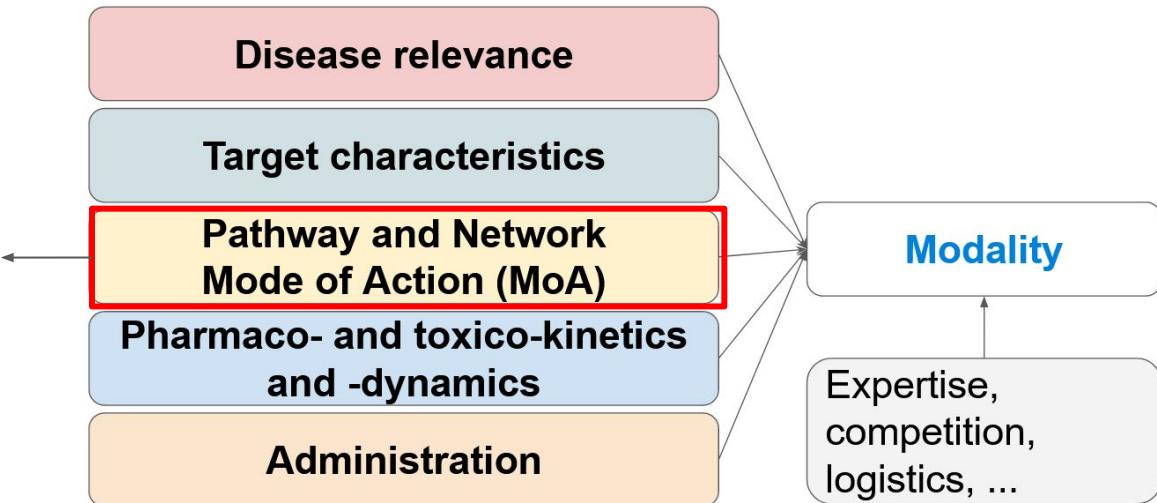
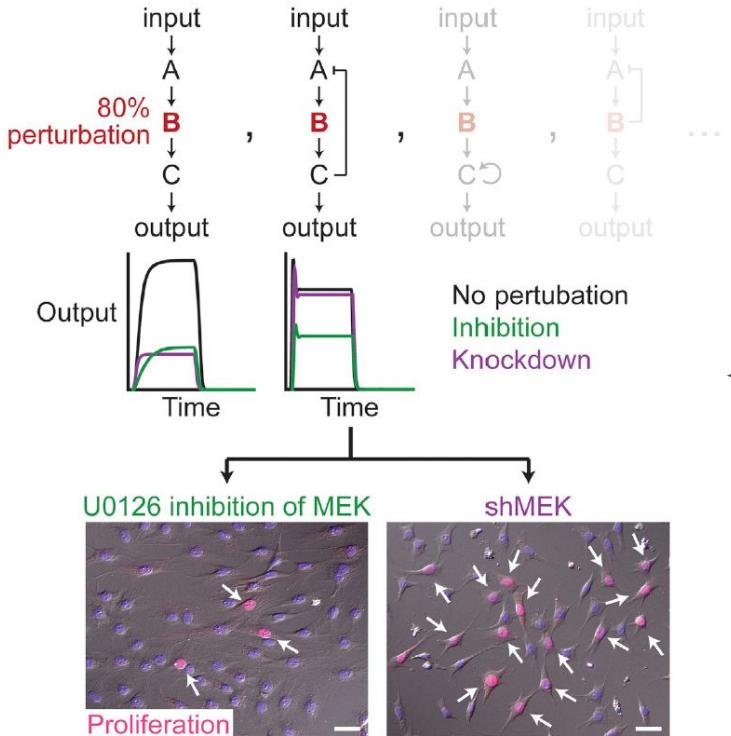
The MAPK/ERK pathway downstream of EGFR signalling



Confirmation of predicted difference of KD and CI



Computational biology may empower our choice of modality



Conclusions

- Given mechanistic understanding of biological processes underlying diseases, we can develop different modalities as therapeutics;
- Mathematical and computational biology
 - (1) helps with molecule design;
 - (2) reveals how drug candidate work and ranks them;
 - (3) contributes to modality selection;

Offline Activities

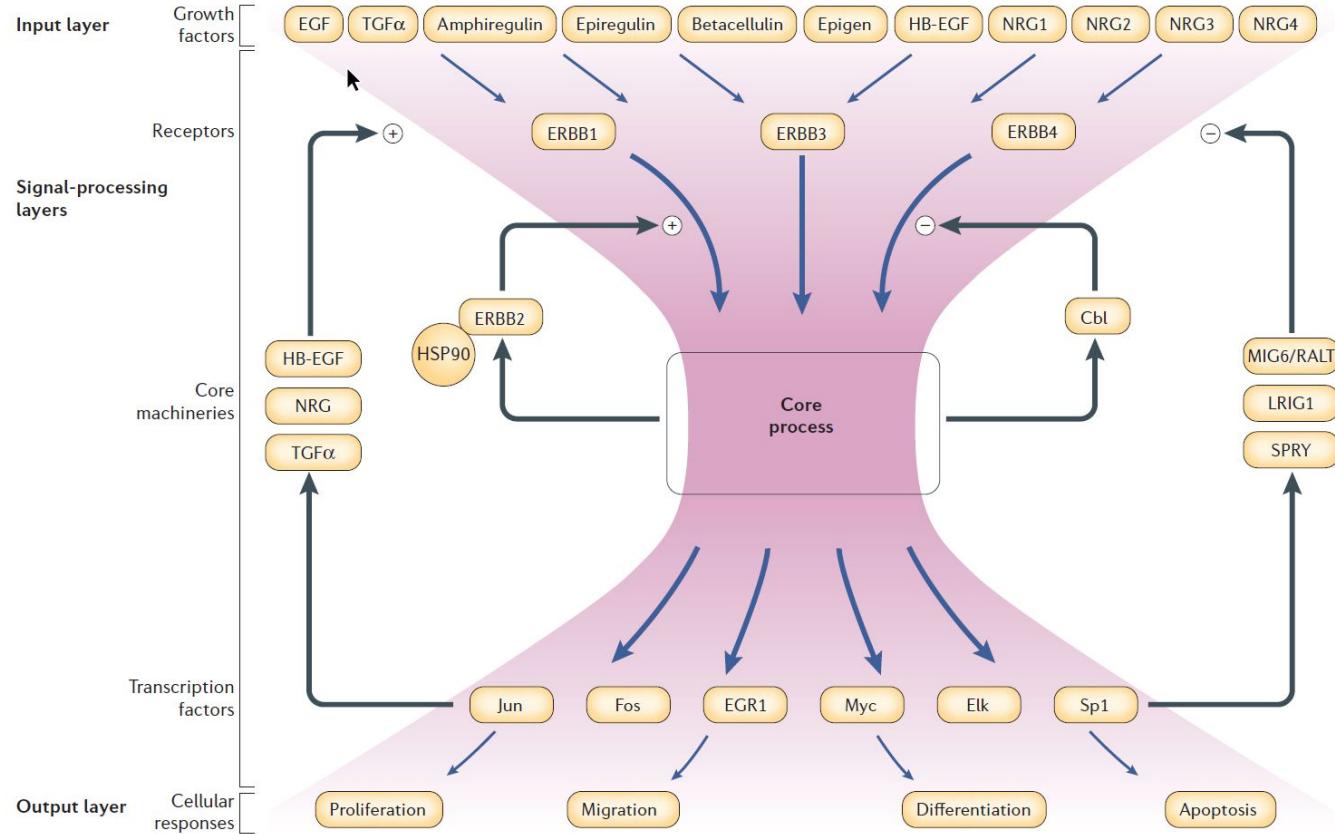
Read about RNA therapies and vaccinations and build your own opinions about them:

- Levin, Arthur A. 2019. “Treating Disease at the RNA Level with Oligonucleotides.” New England Journal of Medicine 380 (1): 57–70. <https://doi.org/10.1056/NEJMra1705346>.
- Oligonucleotides and their discontents by Derek Loewe in *In The Pipeline*:
<https://blogs.sciencemag.org/pipeline/archives/2021/03/24/oligonucleotides-and-their-discontents>
- The next act for messenger RNA could be bigger than covid vaccines, MIT Technology Review 2021, by Antonio Regalado,
<https://www.technologyreview.com/2021/02/05/1017366/messenger-rna-vaccines-covid-hiv/>

Overview of lecture #7

- The Bow-Tie model and why antibodies can achieve wonders
- Antibody and cell-therapy for cancer immunotherapy
- Multispecific drugs: Small molecule: Thalidomide, PROTAC, degraders

The Bow-Tie model of signaling transduction



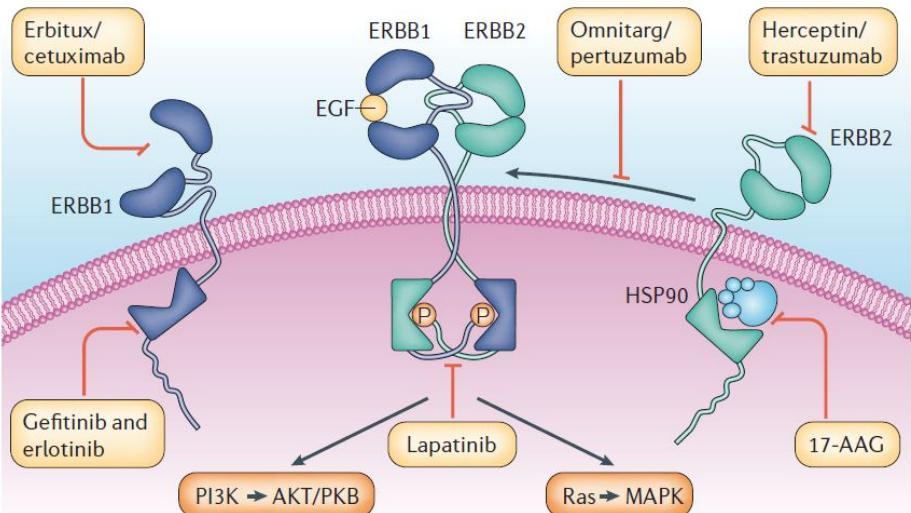
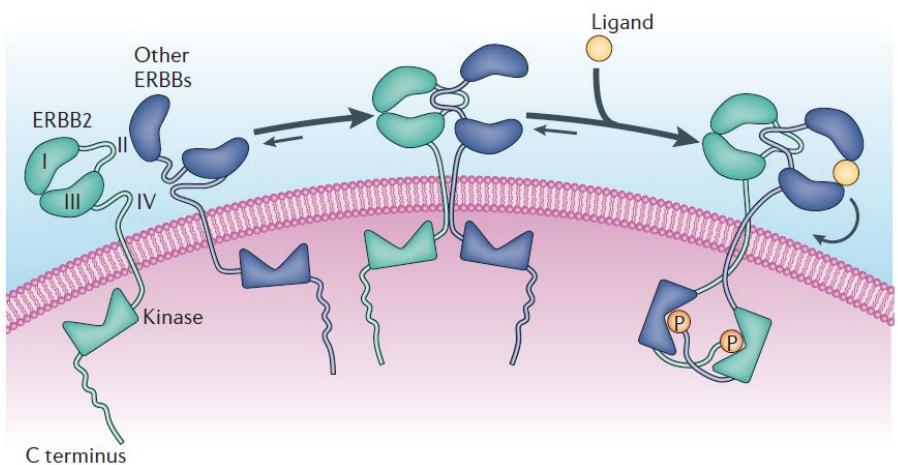
Extracellular
Cell
Membrane

Cytoplasm

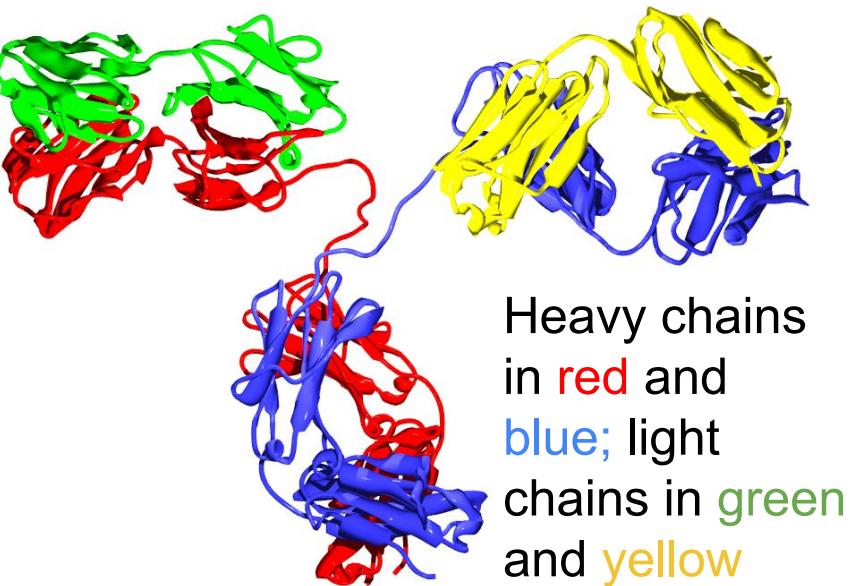
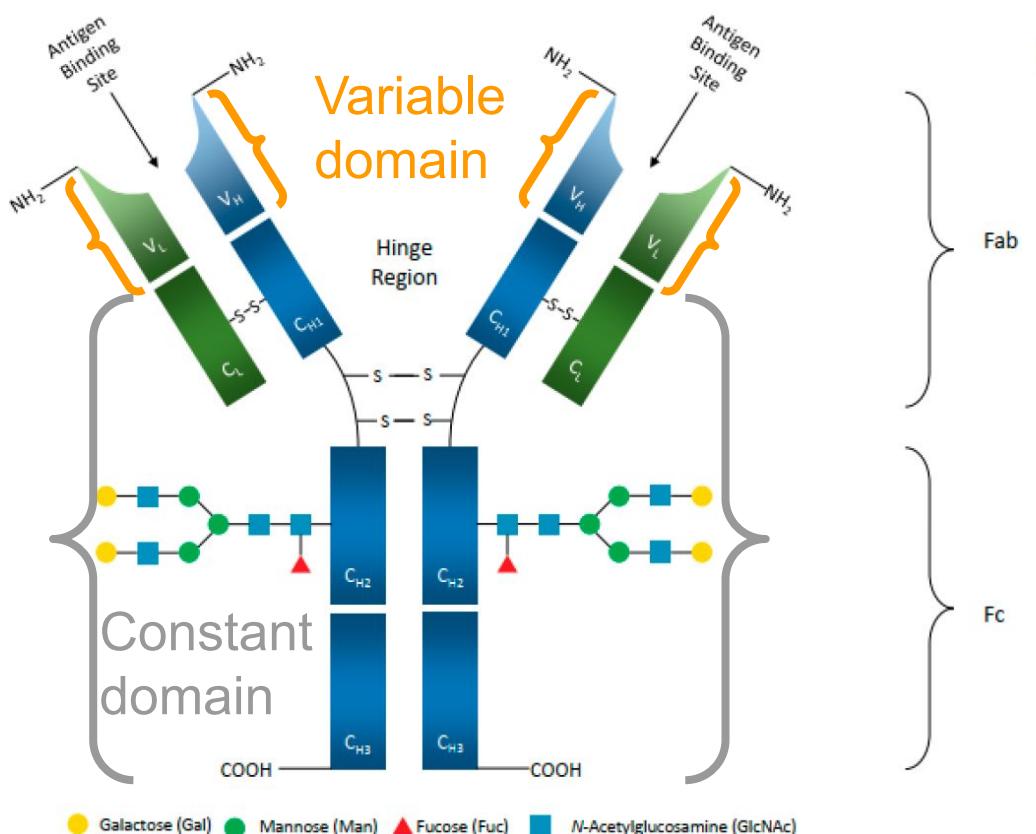
Nucleus

Everywhere

ERBB signaling system and antibody drugs



Structure of antibodies



Fc=fragment crystallizable region

Fab=fragment antigen binding

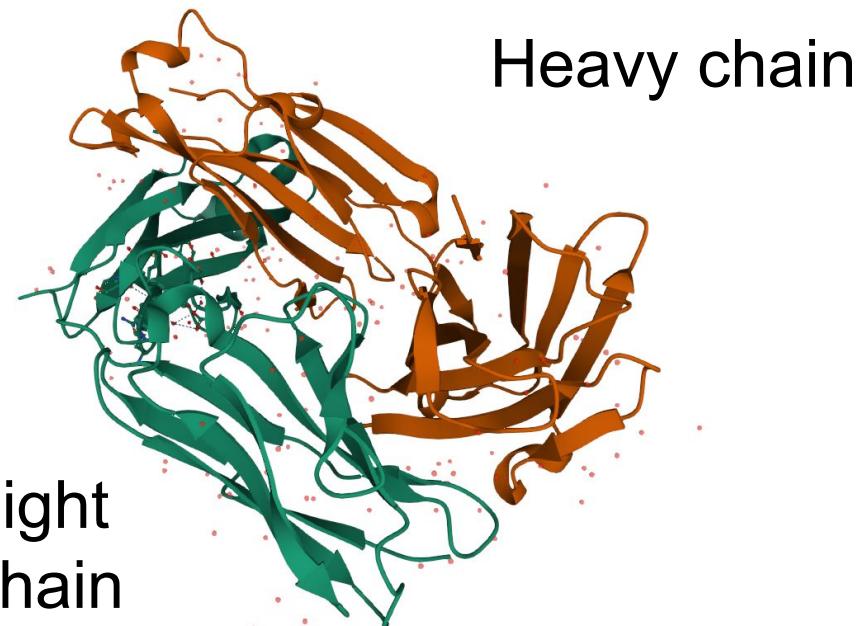
Cetuximab as an example

Variable heavy chain

QVQLKQSGPGLVQPSQSL SITCTVSGF
SLTNYGVHWVRQSPGKGLEWLGVIVSG
GNTDYNTPFTSRLSINKDNSKSQVFFK
MNSLQSNDTAIYYCARALTYYDYEFA
WGQGTLVTVSA

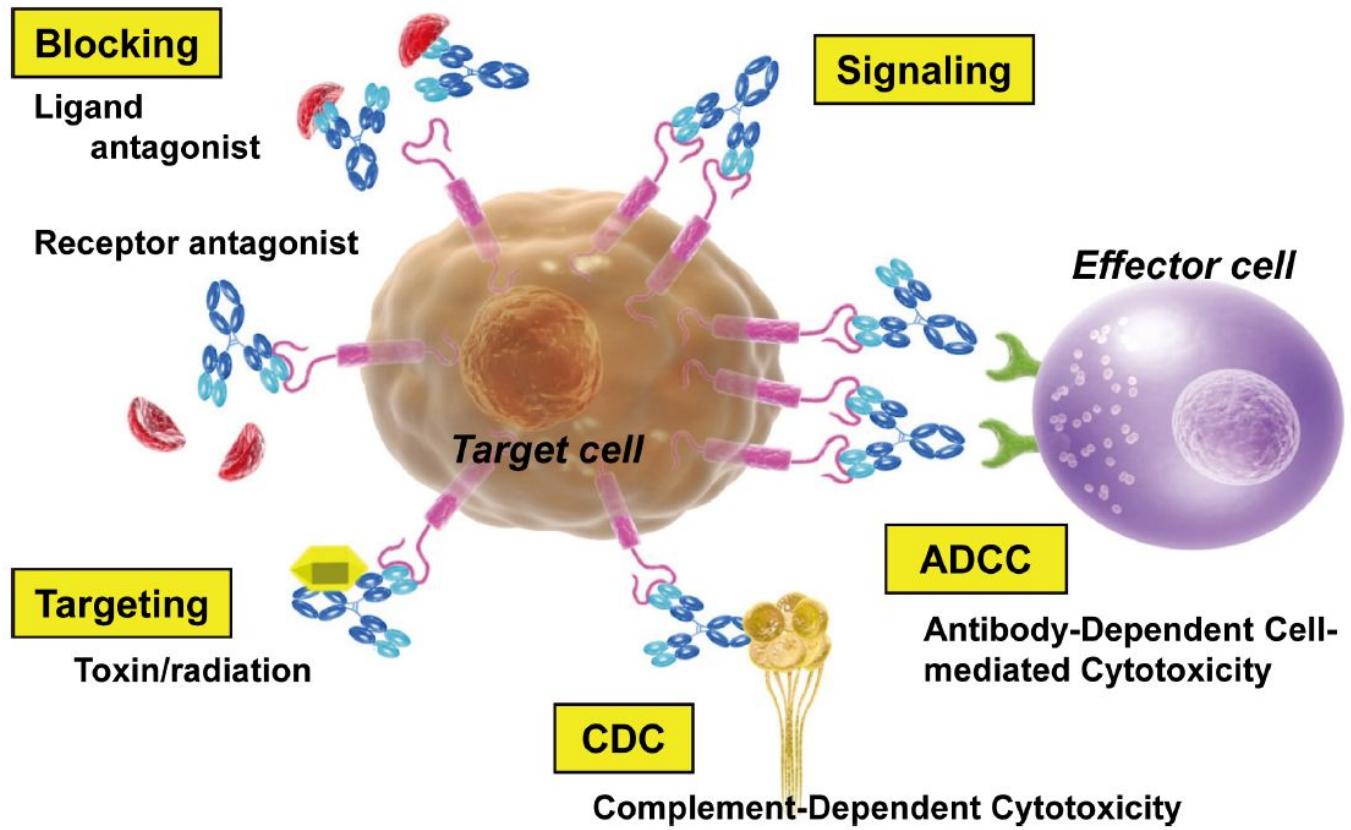
Variable light chain

DILLTQSPVILSVSPGERVS FSCRASQ
SIGTNIHWYQQRTNGSPRLLIKYASES
ISGIPSRFSGSGSGTDFTLSINSVESE
DIADYYCQQNNNWPTTFGAGTKLELK

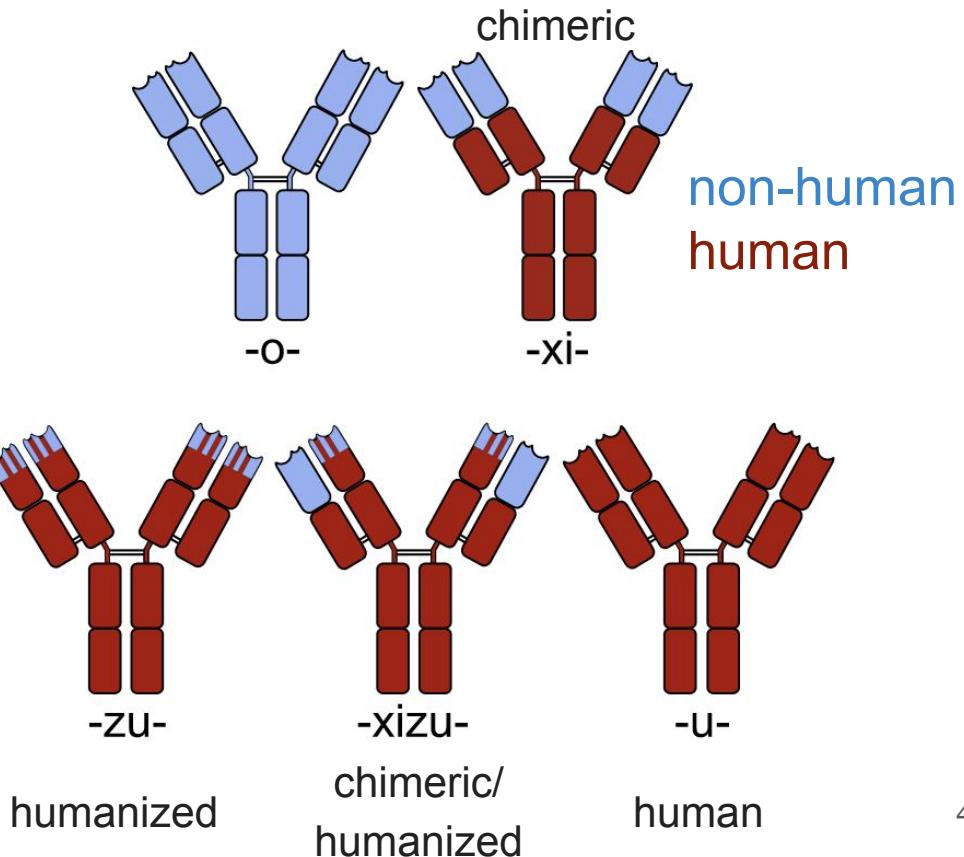
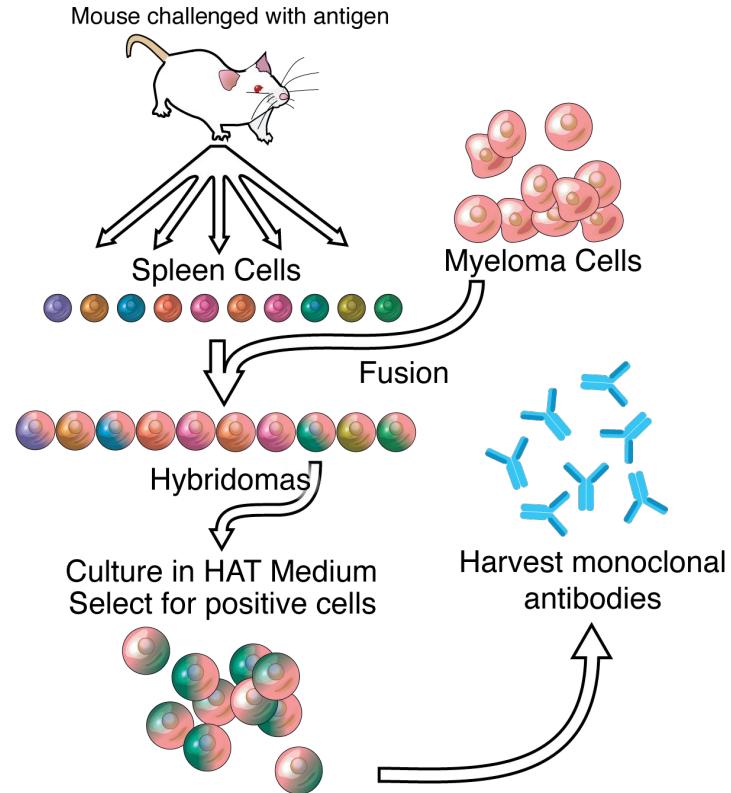


PDB 1YY8

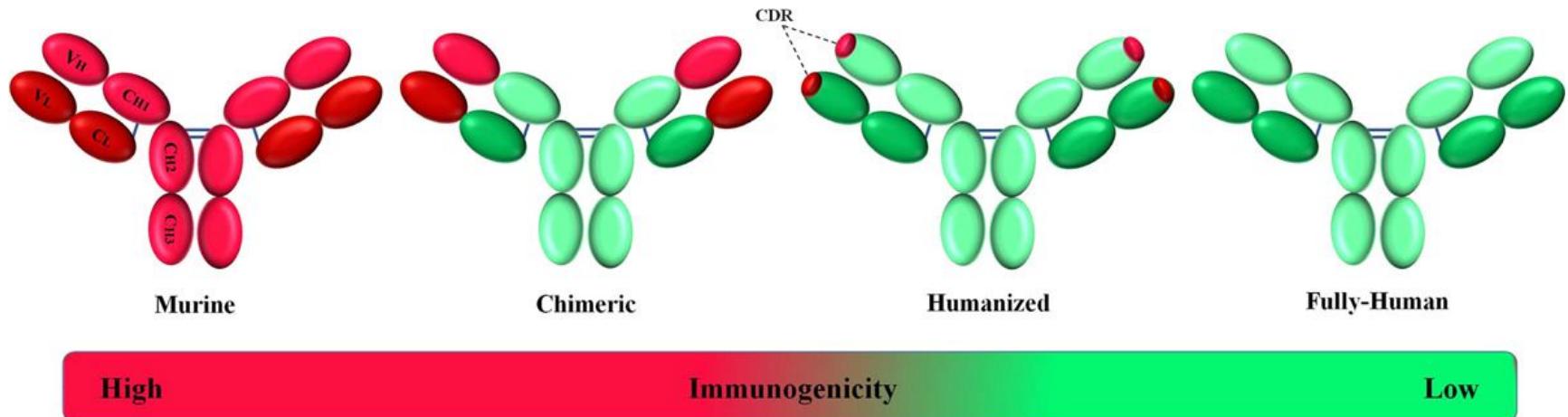
Mechanisms of action of therapeutic antibodies



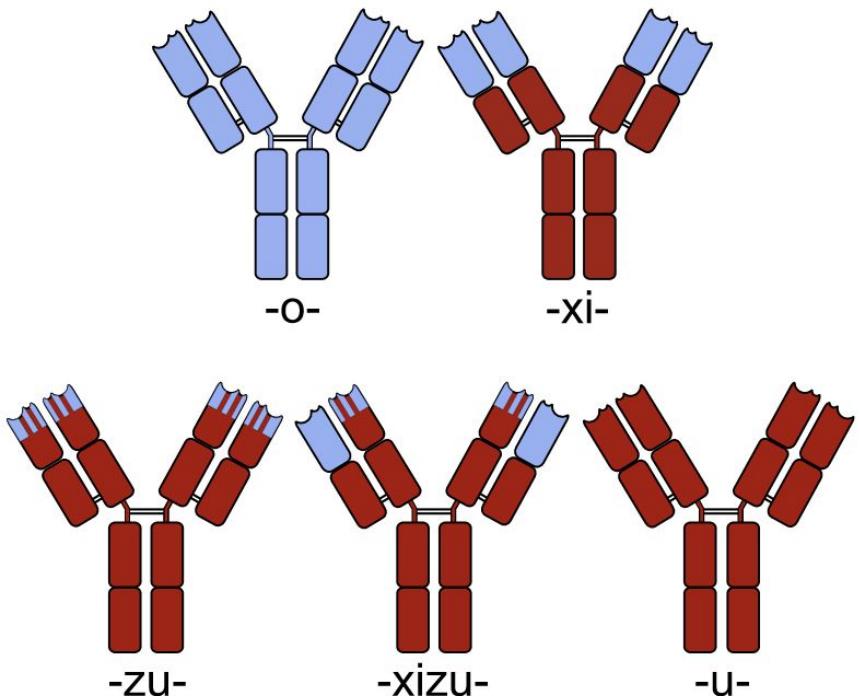
Therapeutic antibody discovery with hybridoma and humanization



Evolution of therapeutic antibodies



Antibody names suggest their types

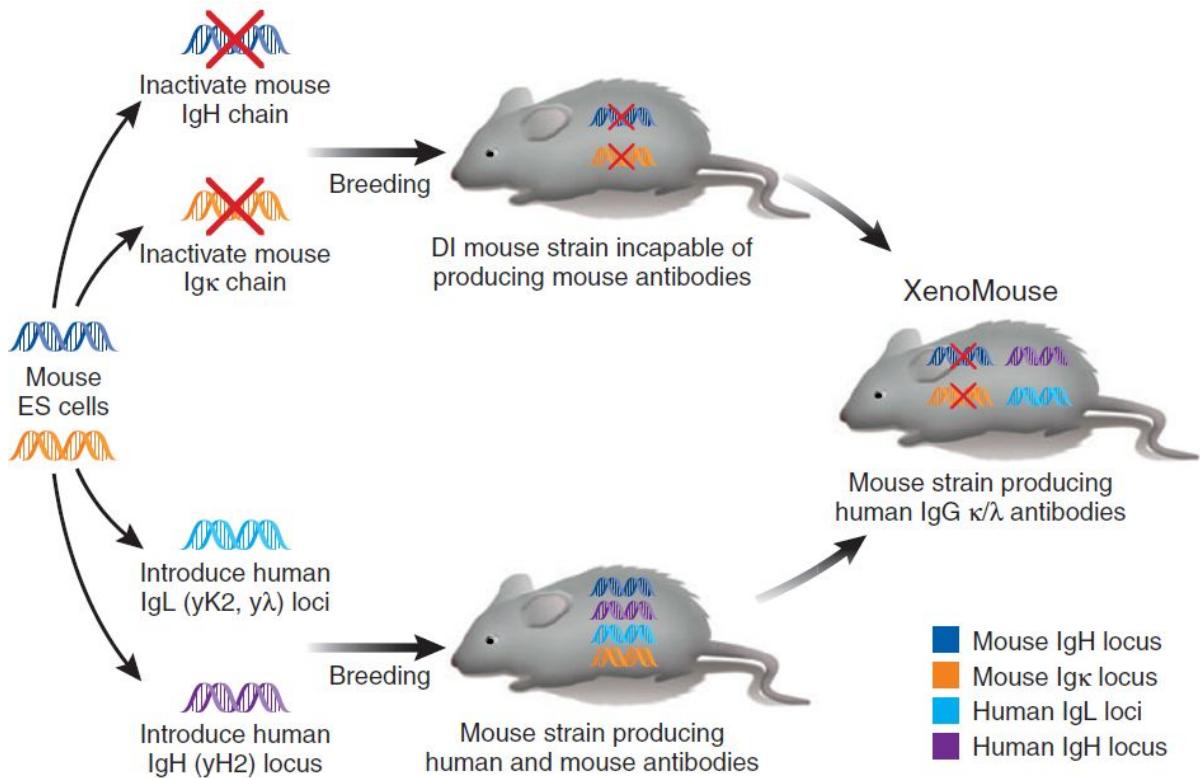


- **Chimeric:** Abiciximab (Ab against platelet aggregation inhibitor)
- **Humanized:** Trastuzumab (HER2)
- **Chimeric/Humanized:** Otelixizumab (CD3, a T lymphocyte receptor)
- **Human:** Adalimumab (TNF-alpha)

Therapeutic antibody discovery with transgenic animals

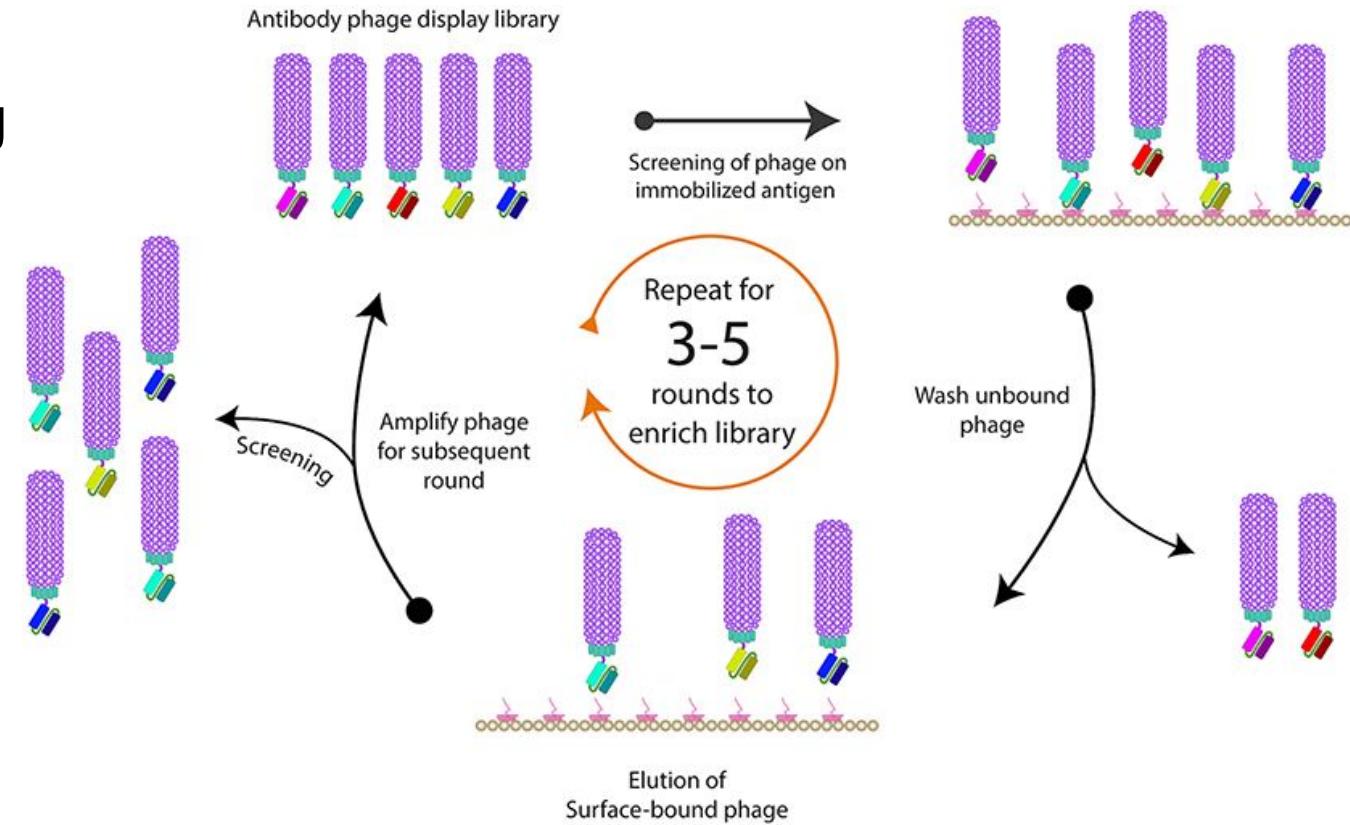
The XenoMouse model, which led to the discovery of panitumumab (Vectibix).

Panitumumab targets EGFR for advanced colorectal cancer.



The principle of phage display

A protein-encoding gene is inserted into the phage coat protein gene, causing the phage to **display** the protein, which can be screened *in vitro* iteratively.



Antibody discovery with phage display

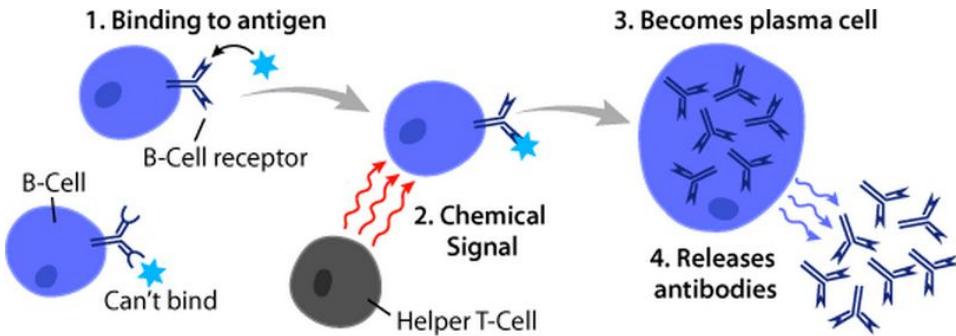
Convalescent or patient blood plasma

RNA → cDNA

Copy antibody-coding sequences

Paste sequences into phage coats

Copy/Paste: PCR and cloning



Aim: Reverse-engineering existing antibodies

Antibody selection

Selected challenges of antibody discovery

- *Lack of quantitative rules of developability*
- Immunogenicity
- Computational modelling and design of antibodies

Biophysical properties of clinical-stage antibodies

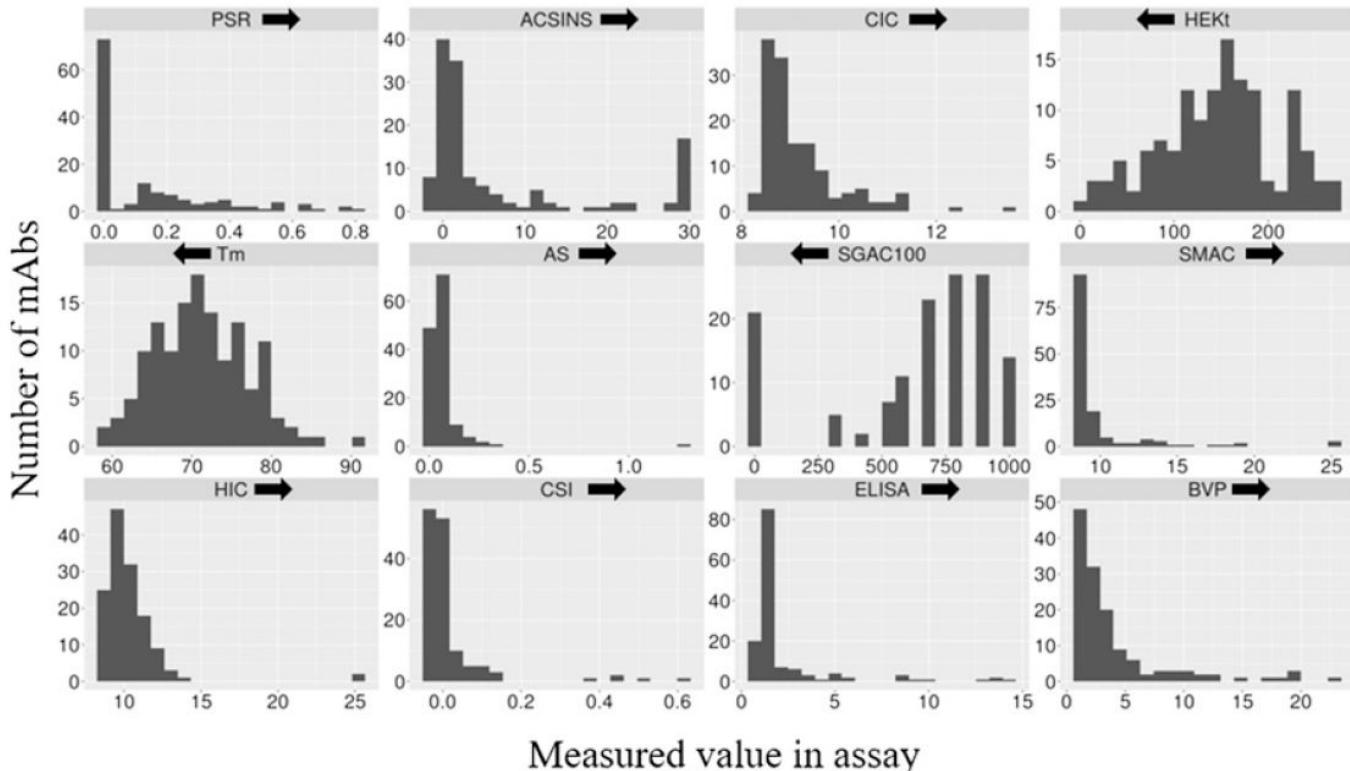
Name	Light chain class	Type	Original mAb Isotype or Format	Clinical Status	Phage ^c	Year Name Proposed	Source Detailed ^a				
			IgG2	Phase 2	No.	2013	VH	VL	LC Class	Source	
abituzumab	kappa	zeta									
abrilumab	kappa	lambda									
adalimumab	kappa	lambda									
alemtuzumab	kappa	lambda									
alirocumab	kappa	lambda									
anifrolumab	kappa	lambda									

Name	HEK Titer (mg/L)	Fab Tm by DSF (°C)	SGAC-SINS AS100 ((NH4)2SO4 mM)	HIC Retention Time (Min) ^a	SMAC Retention Time (Min) ^a	Slope for Accelerated Stability	Poly-Specificity Reagent (PSR) SMP Score (0-1)	Affinity-Capture Self-Interaction Nanoparticle Spectroscopy (AC-SINS) Δλmax (nm) Average		CIC Retention Time (Min)	CSI-BLI Delta Response (nm)	ELISA	BVP ELISA
								Interaction Nanoparticle Spectroscopy (AC-SINS) Δλmax (nm)	Average				
abituzumab	89.6	75.5	900.0	9.2	8.7	0.06	0.17	1.5	8.6	0.00	1.14	2.72	
abrilumab	100.2	71.0	900.0	9.4	8.7	0.03	0.00	-0.9	8.4	-0.02	1.12	1.82	
adalimumab	134.9	71.0	900.0	8.8	8.7	0.05	0.00	1.1	8.9	-0.01	1.08	1.49	
alemtuzumab	144.7	74.5	1000.0	8.8	8.7	0.06	0.00	-0.8	8.5	-0.02	1.16	1.46	
alirocumab	69.2	71.5	900.0	9.0	8.7	0.03	0.00	1.2	8.8	-0.01	1.20	2.18	
anifrolumab	82.0	62.5	700.0	8.8	8.6	0.07	0.00	-0.6	8.5	-0.02	1.16	1.62	
atezolizumab	164.1	73.5	300.0	13.4	19.3	0.06	0.07	15.0	10.8	0.06	1.29	6.20	
bapineuzumab	151.1	73.0	1000.0	8.9	8.7	0.07	0.00	-0.7	8.6	0.06	1.21	3.55	
basiliximab	107.5	60.5	0.0	9.6	8.6	0.05	0.40	28.8	9.4	0.00	1.20	2.14	
bavituximab	45.1	59.5	0.0	11.5	12.7	0.04	0.56	29.9	11.4	-0.01	1.32	1.69	
belimumab	10.5	60.0	800.0	10.5	9.3	0.13	0.00	0.8	8.6	-0.03	3.61	12.23	

Twelve different biophysical assays

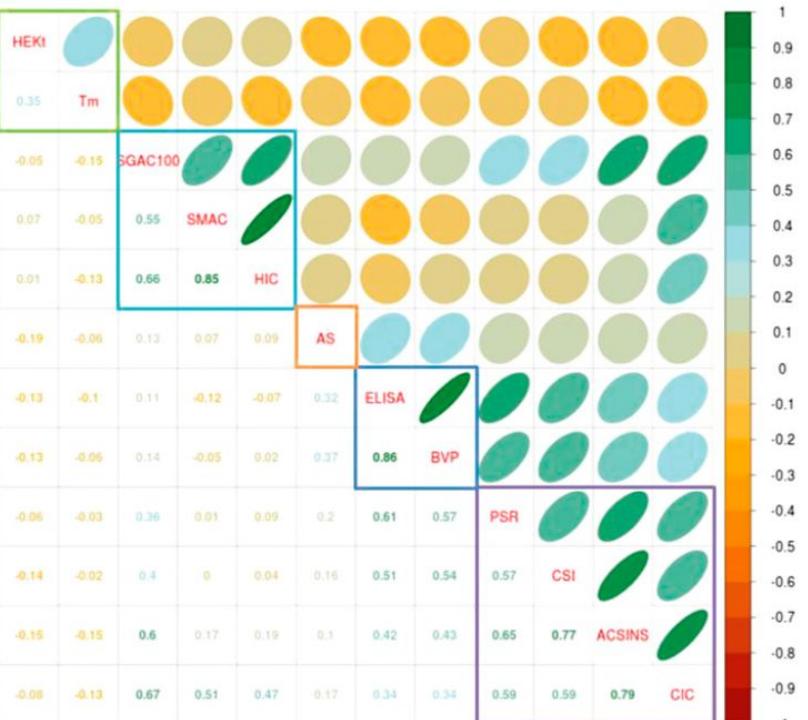
Code	Name	Purpose	Code	Name	Purpose
AC-SINS	Affinity-capture self-interaction nanoparticle spectroscopy	Self-interaction	HEK	Expression titer in HEK cells	Expression
CSI	Clone self-interaction by biolayer interferometry	Self-interaction	Tm	Melting temperature	Thermostability
PSR	Poly-specificity reagent	Cross-interaction	HIC	Hydrophobic interaction chromatography	Species separation and analysis
BVP	Baculovirus particle	Cross-interaction	SAGC-SINS	salt-gradient affinity-capture self-interaction nanoparticle spectroscopy	Species separation and analysis
CIC	Cross-interaction chromatography	Cross-interaction	SMAC	standup monolayer adsorption chromatography	Developability
ELISA	Enzyme-linked immunosorbent assay with commonly used antigens	Cross-interaction	AS	Size-exclusion chromatography in accelerated stability	Stability

Distribution of results from biophysical assays for 137 monoclonal antibodies

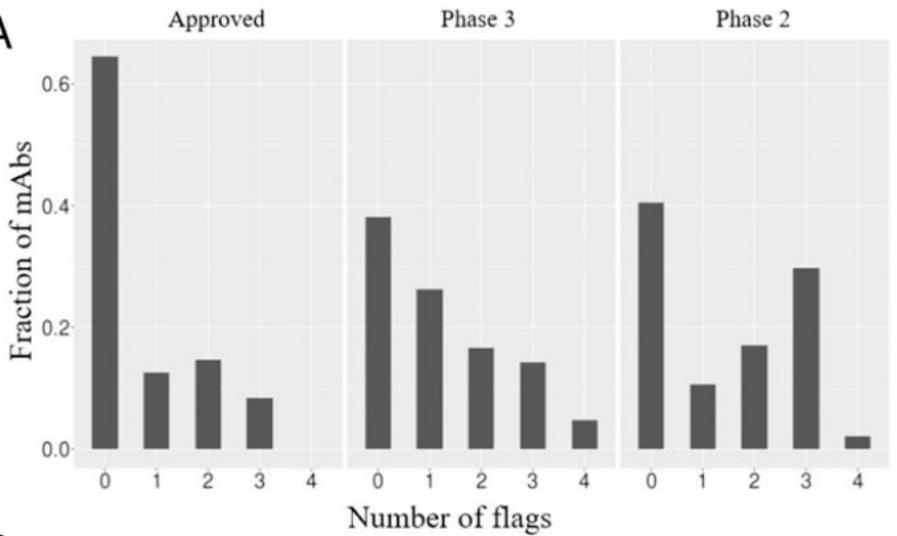
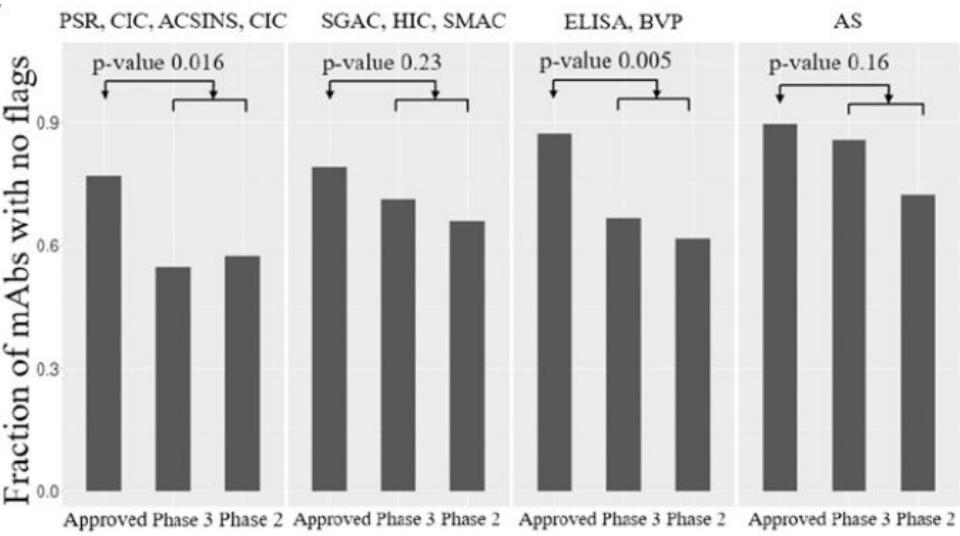


Unsupervised clustering analysis reveals related assays

Group	Assay	Worst 10% threshold
Group 1	PSR	0.27 ± 0.06
	ACSINS	11.8 ± 6.2
	CSI	0.01 ± 0.02
	CIC	10.1 ± 0.5
Group 2	HIC	11.7 ± 0.6
	SMAC	12.8 ± 1.2
	SGAC-SINS	370 ± 133
	BVP	4.3 ± 2.2
Group 3	ELISA	1.9 ± 1.0
	AS	0.08 ± 0.03



Approved antibodies tend to have fewer flags

A

B


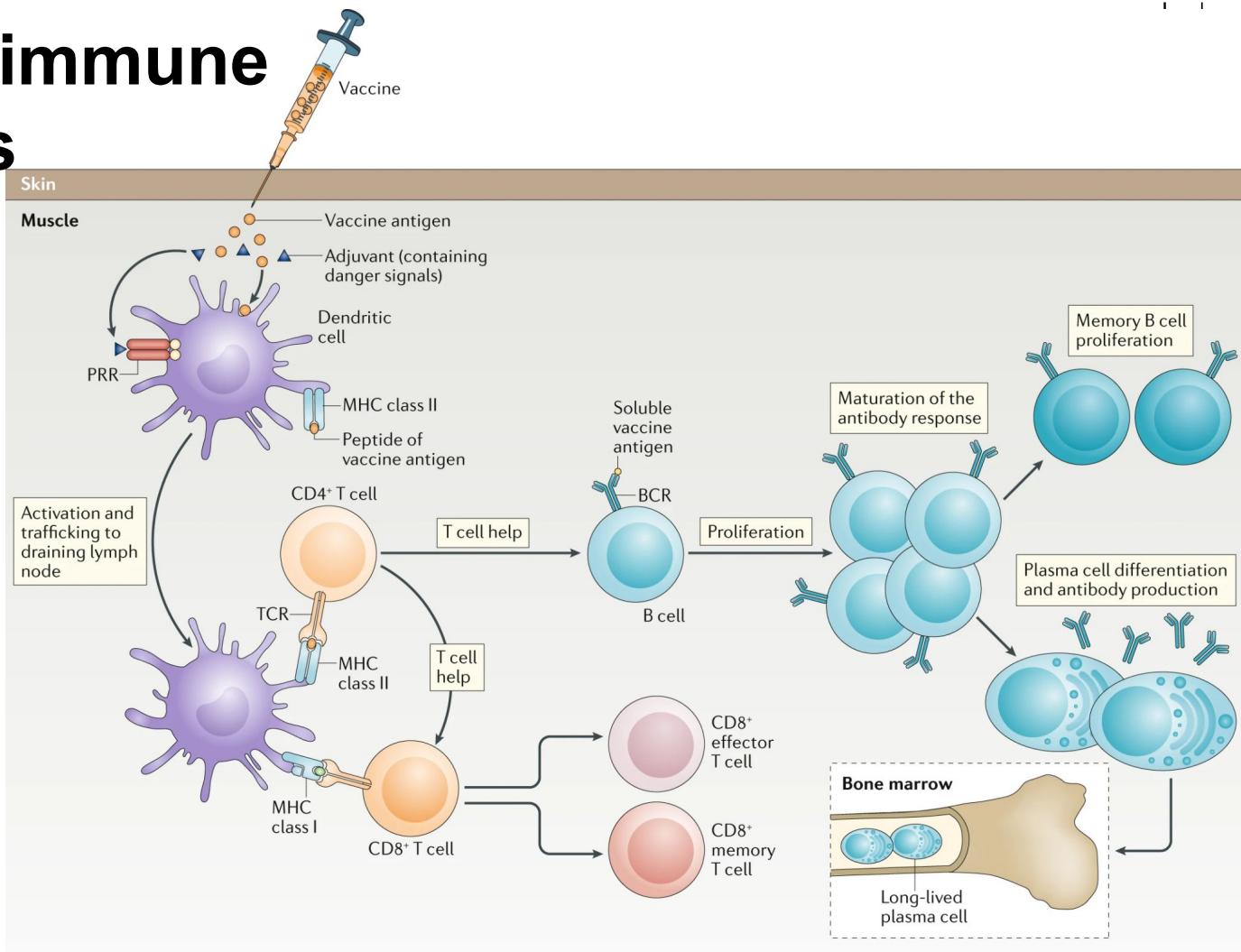
Interim summary

- Therapeutic antibody discovery with (1) hybridoma, (2) with transgenic animals, and (3) phage display;
- Systematic data generation and analysis of clinical-stage antibodies reveal (1) correlations between biophysical measurements and (2) negative association between development stage and some measurements.

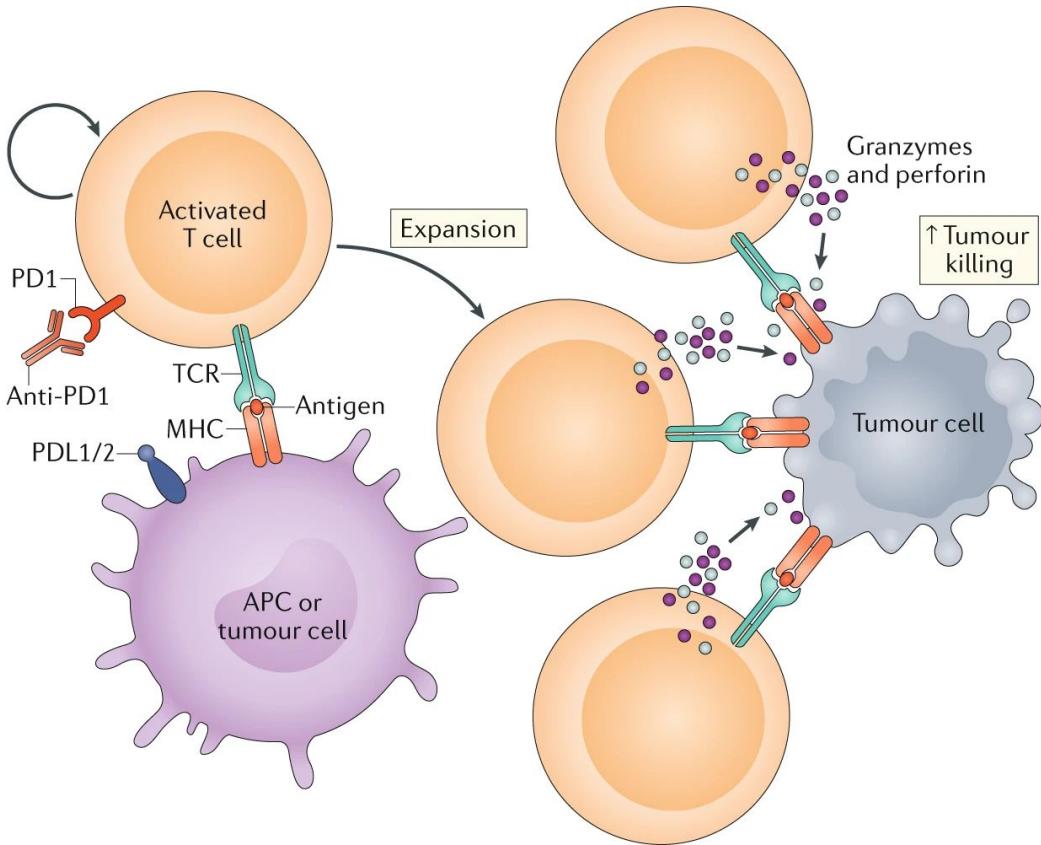
How vaccine (immune system) works

Key players include:

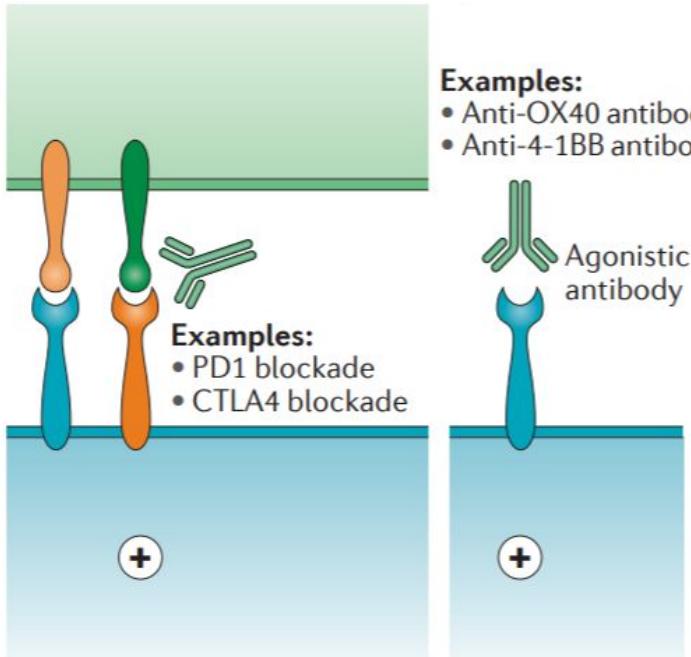
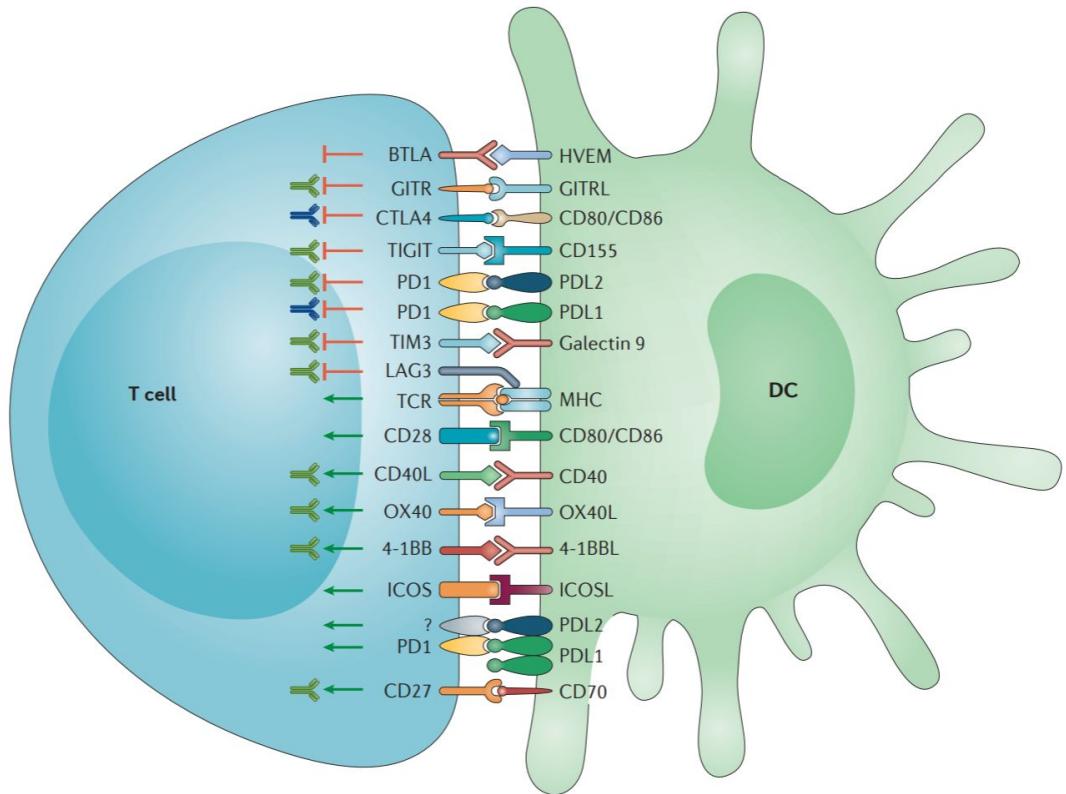
1. dendritic cells
2. T cells
3. B cells



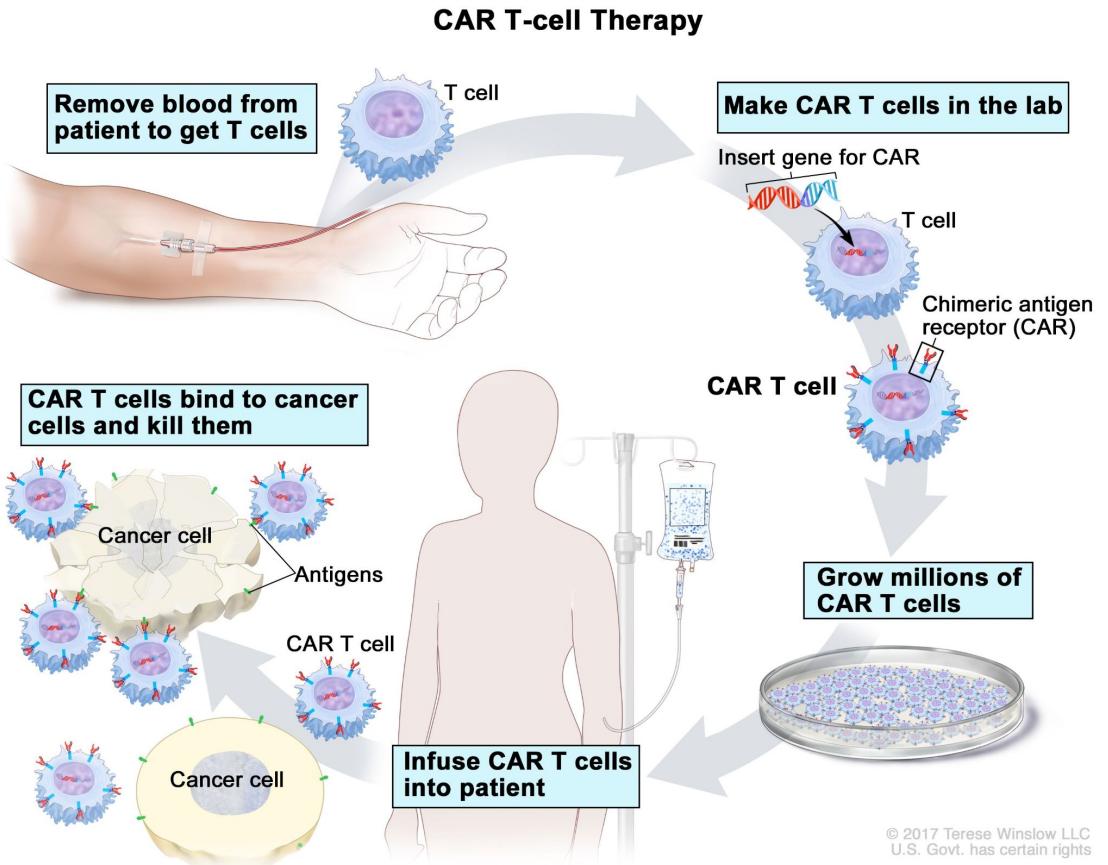
Teamwork of T cells and antigen-presenting cells (APC) to kill tumour cells



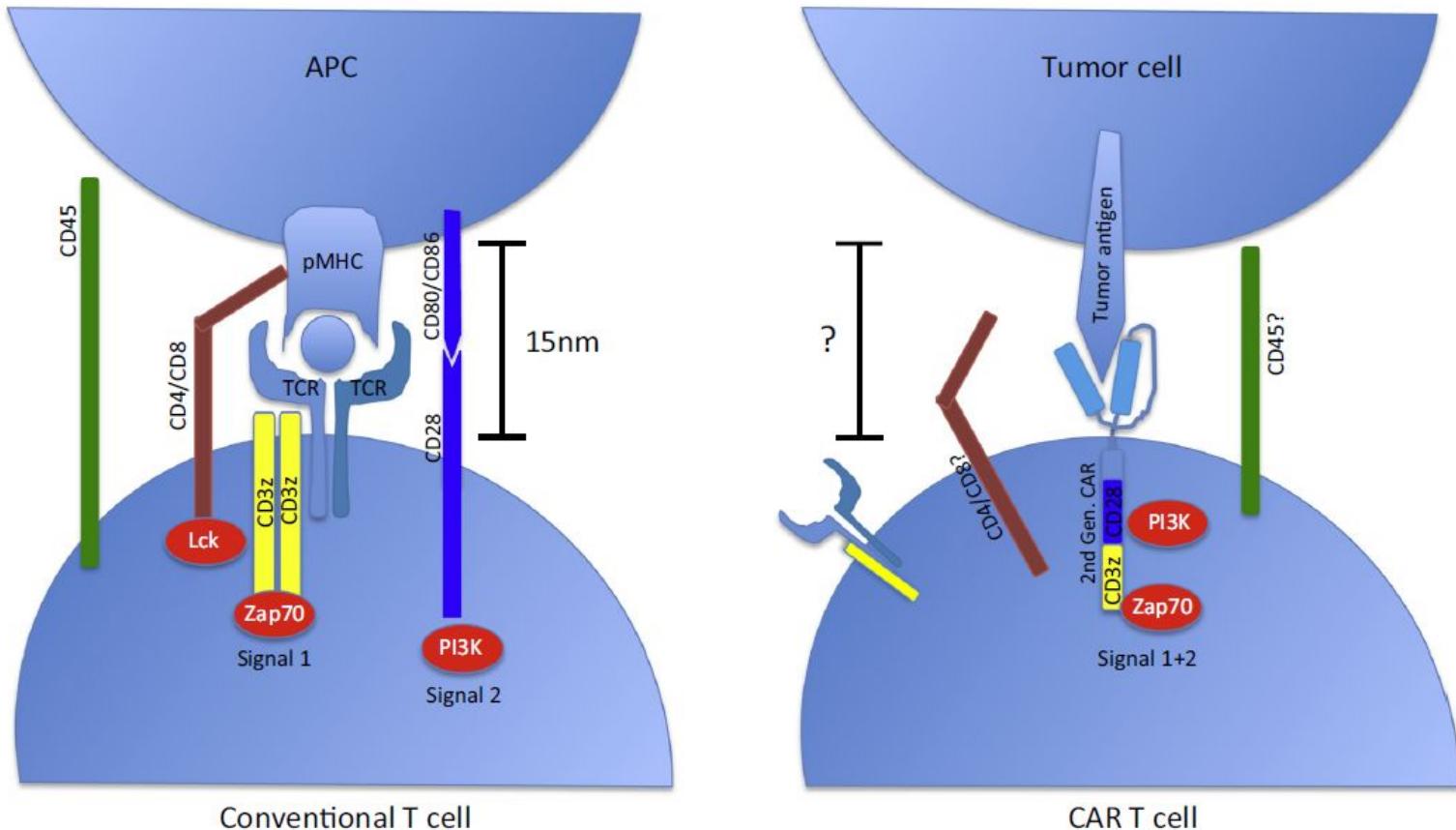
Immune checkpoints as drug targets

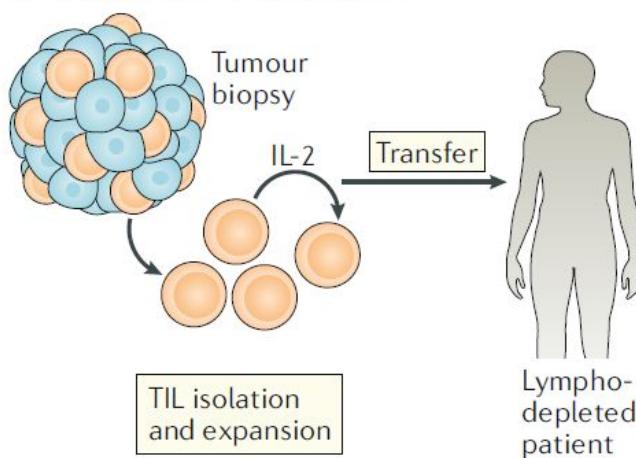
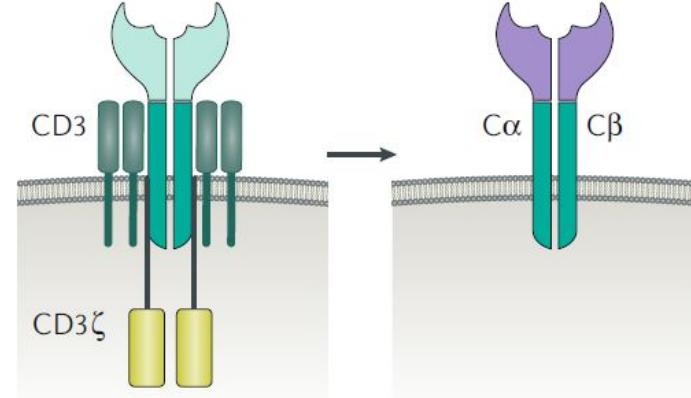
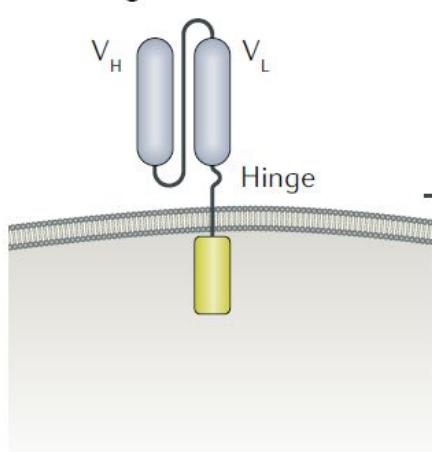
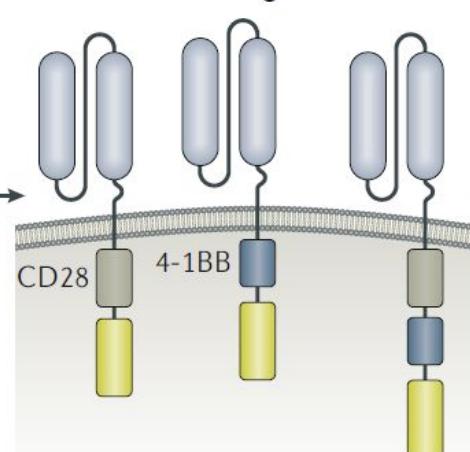
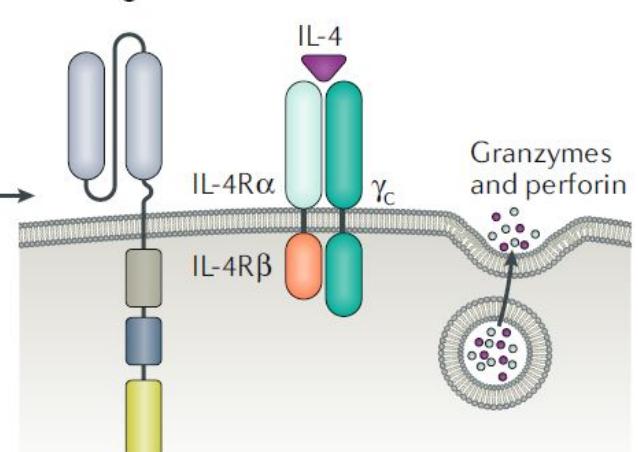


CAR (Chimeric Antigen Receptor) T-Cell Therapy

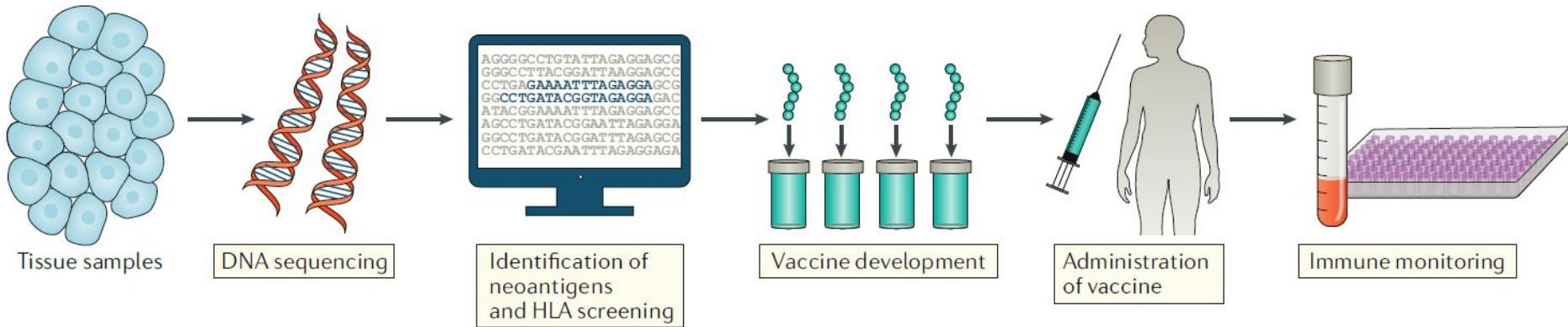


Signaling of conventional and CAR T cells



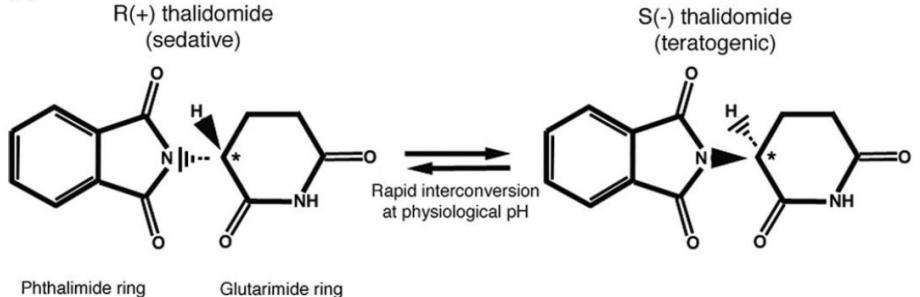
a TAA-specific T cell transfer

b Physiological TCR-CD3 complex

Recombinant TCR
c First-generation CAR

Second- and third-generation CARs

Fourth-generation CAR


Towards personalized vaccine development



The Tragedy of teratogenic S(-) thalidomide in 1950s

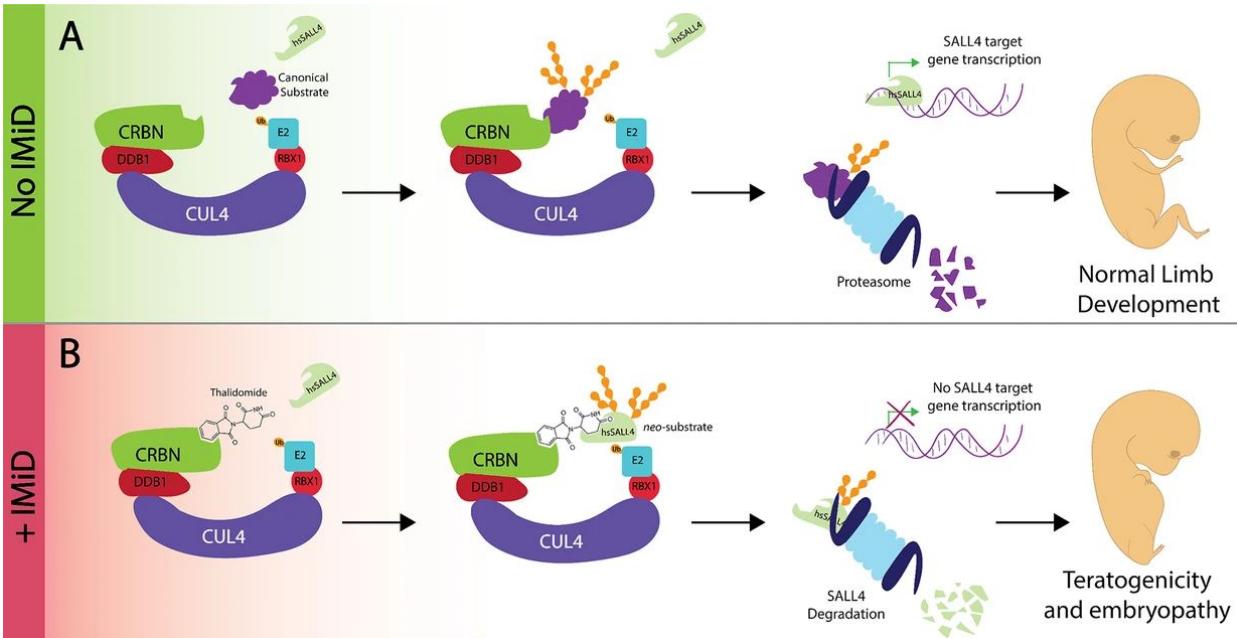
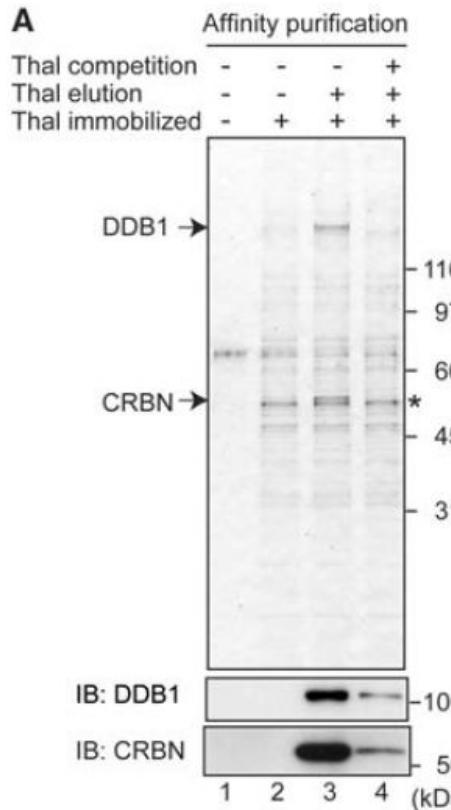
A



B

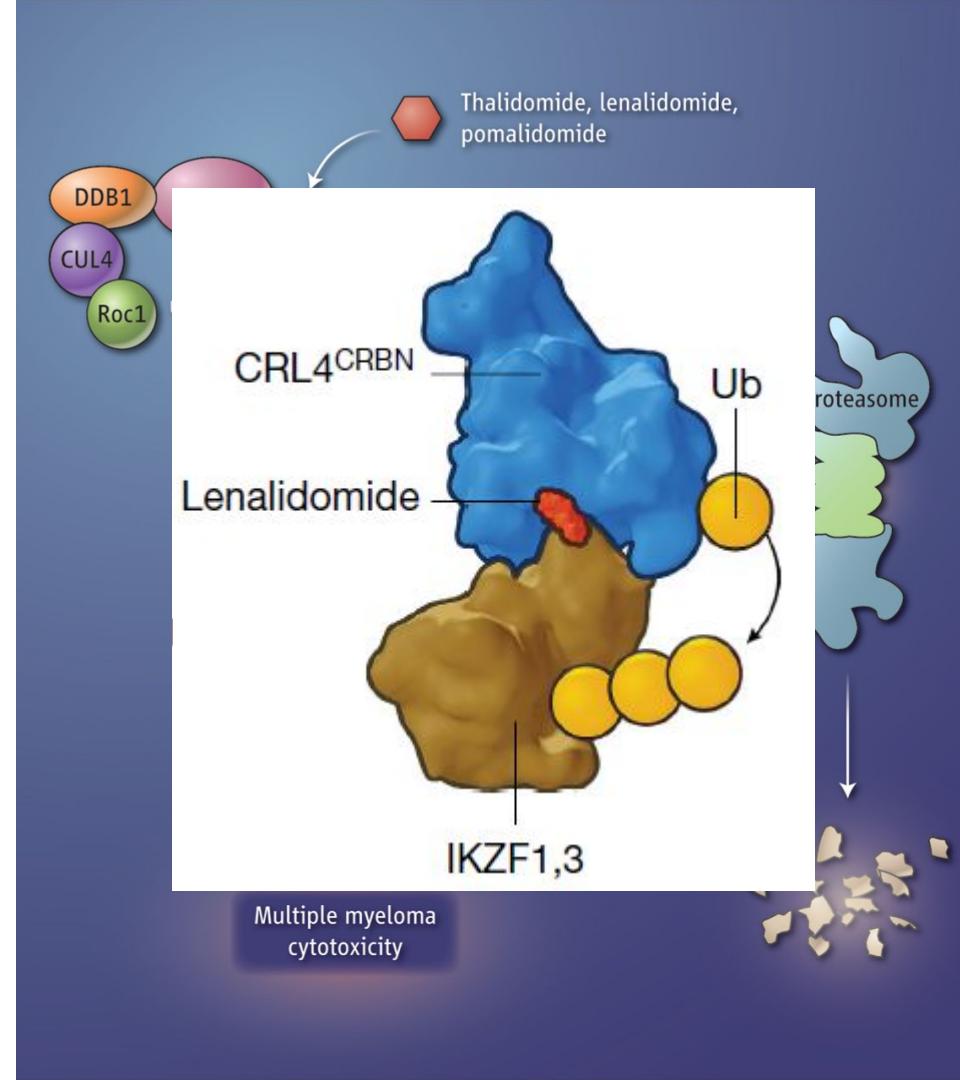


Molecular basis of the teratogenicity of thalidomide reported in 2010



The same mechanism is responsible for efficacy against blood cancers

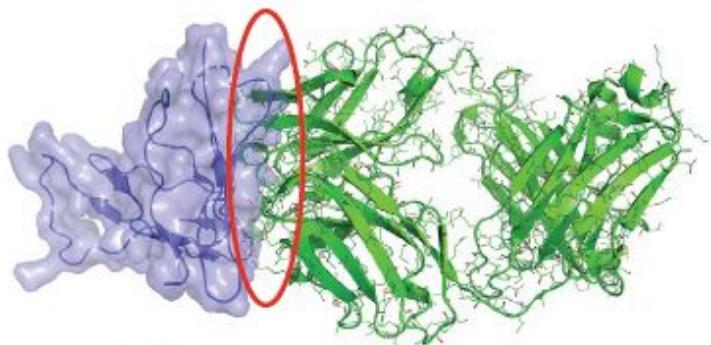
Thalidomide and derivatives bring proteins IKZF1 and IKZF3 close to E3 ubiquitin ligase, leading them to be degraded.



Multispecific Drug Use or Target Interactions

b Conventional drug:

- Forms 1 drug–target interface
- Can act throughout body
- Only works if its binding to target alters function of target



IL-2R α

Basiliximab

c Obligate multispecific drug:

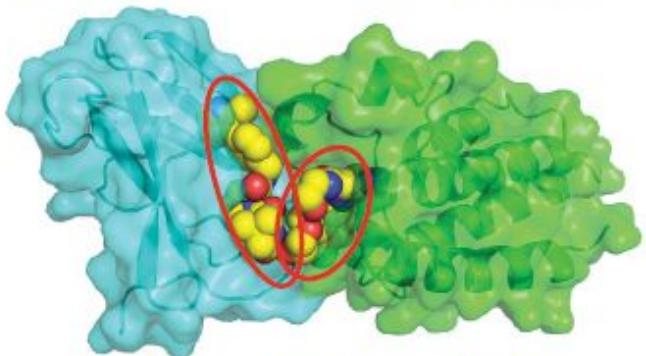
- Forms 2 or more drug–target interfaces

Class 1 ‘tetherbodies’

- Enrich drug at relevant site of action

Class 2 ‘matchmakers’

- Link drug to a biological effector

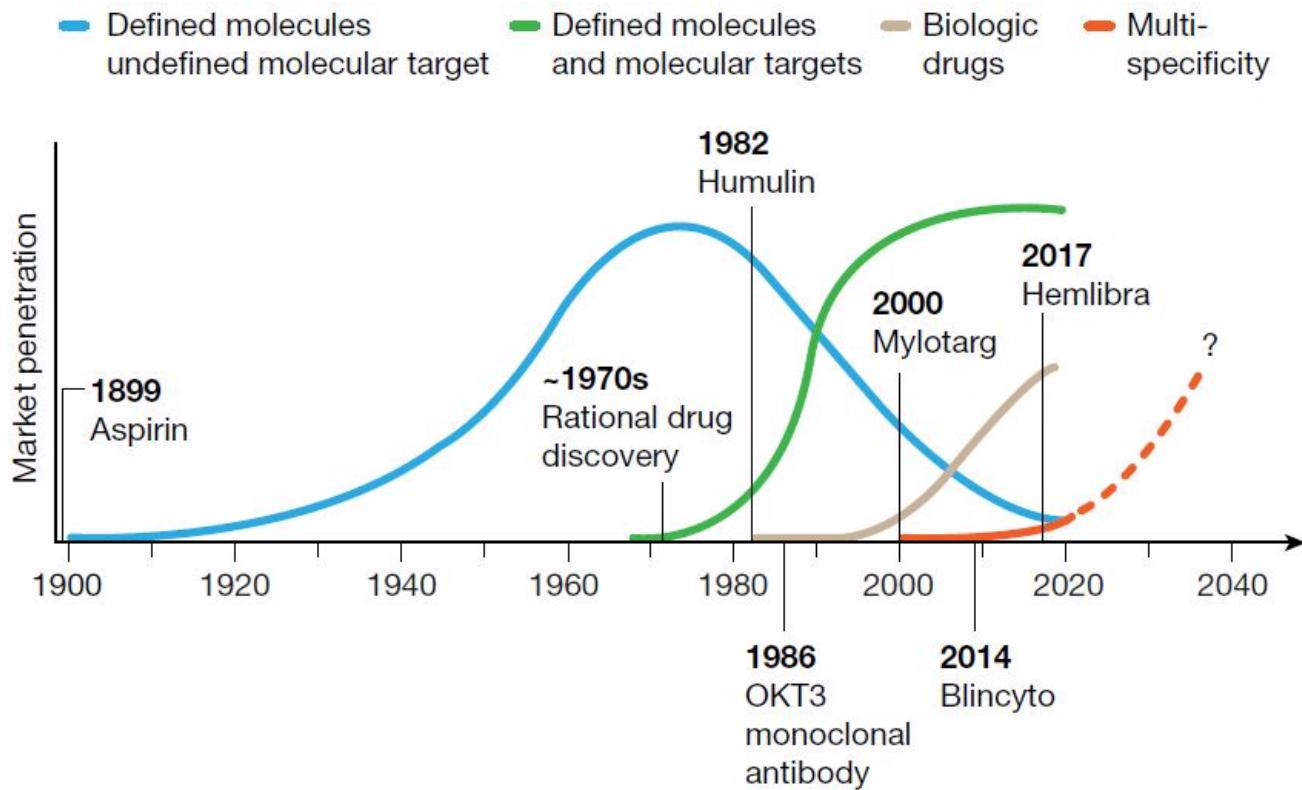


VHL

MZ1

BRD4 BD2

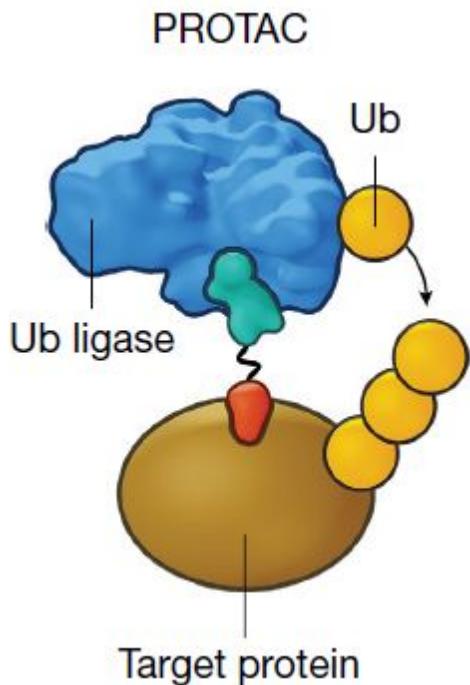
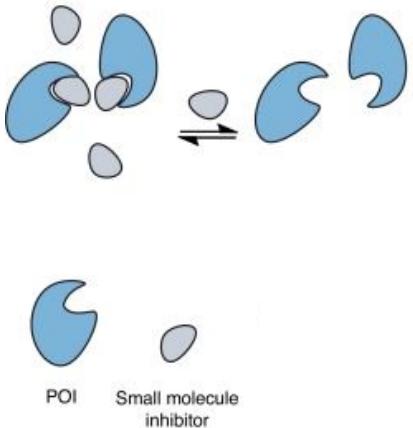
Paradigm shifts or paradigm expansions?



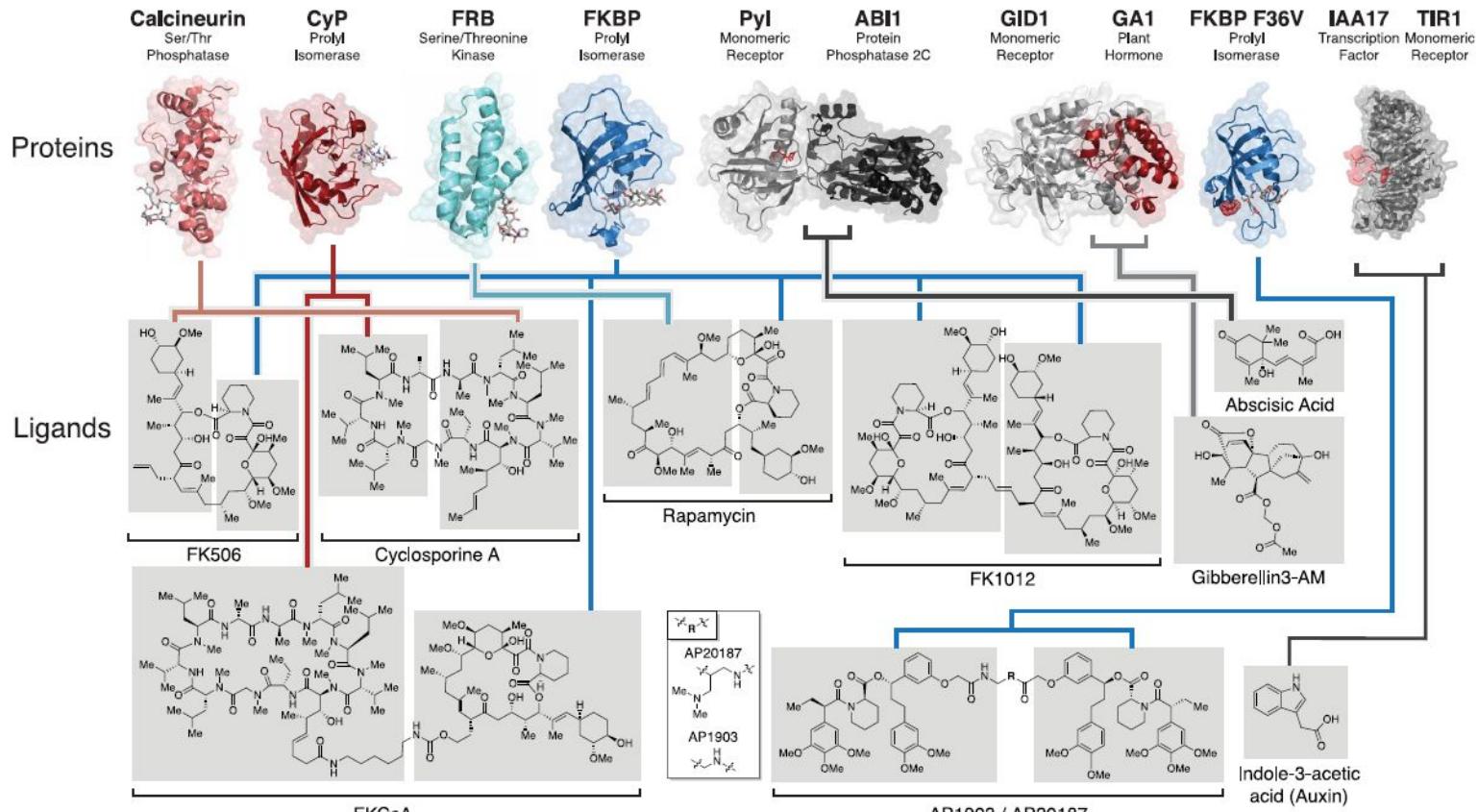
PROteolysis TArgeting Chimera (PROTAC)

(a) Occupancy-driven pharmacology

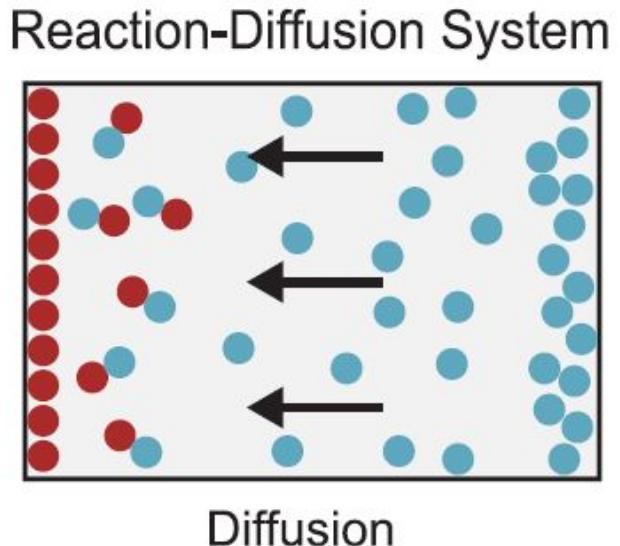
Protein function is modulated *via* inhibition



Chemically induced proximity

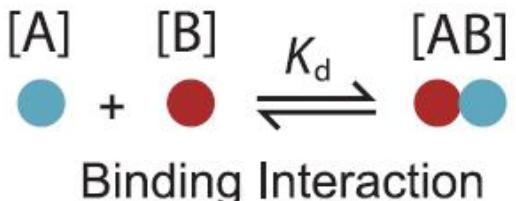


A reaction-diffusion model



$$\frac{\partial u}{\partial t} = D \frac{\partial^2 u}{\partial x^2} + ku$$

Diffusion Binding



x : position

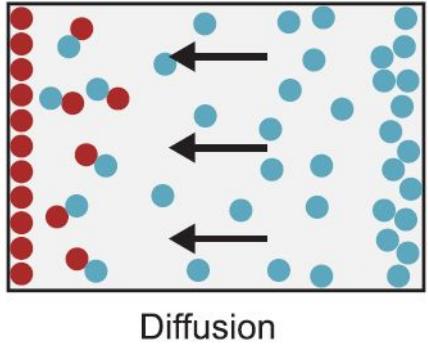
u : product concentration

t : time

The diffusion term follows *Fick's second law of diffusion*; the binding term describes the reaction

Kinetic and thermodynamic contributions of chemically induced proximity

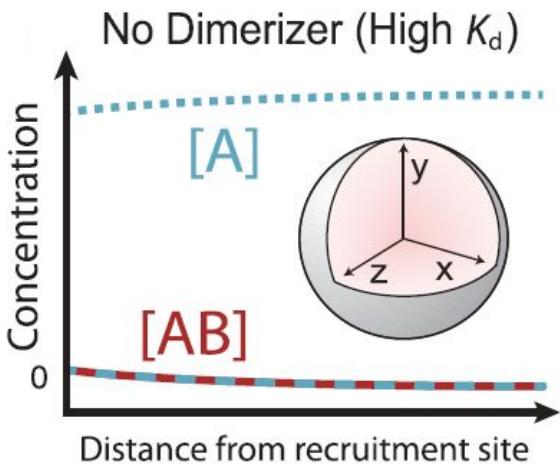
Reaction-Diffusion System



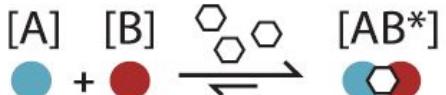
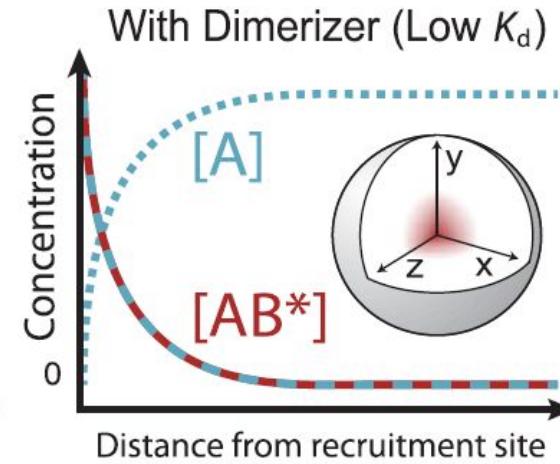
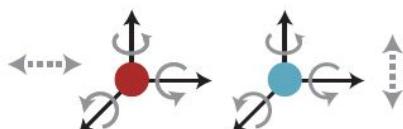
$$\frac{\partial u}{\partial t} = D \frac{\partial^2 u}{\partial x^2} + ku$$



Binding Interaction

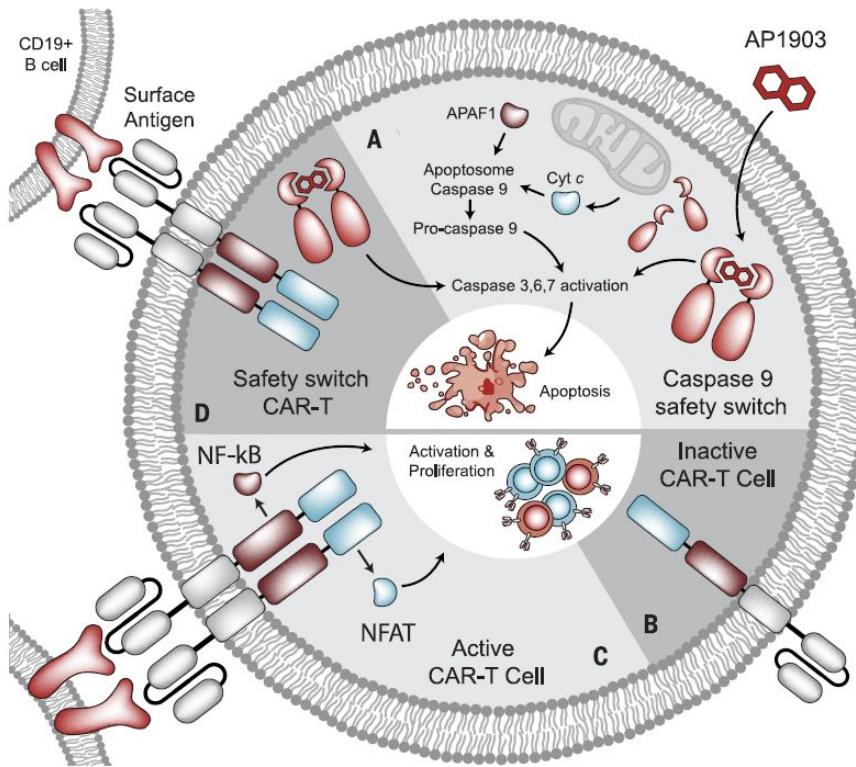


Freely Diffusing



Chemically induced proximity as ‘safety switch’ for cell therapy

- Too many or too active CAR-T cell smay induce serious side effects (cytokine release syndrome, B cell aplasia, etc.)
- *Bioinert* small molecules (AP1903 in this case) can be used as ‘safety switch’ to kill transplanted CAR-T cells.



Conclusions

- *Given mechanistic understanding of biological processes underlying diseases, we can develop different modalities as therapeutics: small molecules, antisense oligonucleotides, antibodies, cell therapies, multispecific drugs, etc.*
- Mathematics and computational biology supports *disease understanding, molecule design, prioritises drug candidates, and contributes to modality selection;*

References

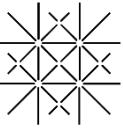
1. Valeur, Eric, Stéphanie M. Guéret, Hélène Adihou, Ranganath Gopalakrishnan, Malin Lemurell, Herbert Waldmann, Tom N. Grossmann, and Alleyn T. Plowright. 2017. "New Modalities for Challenging Targets in Drug Discovery." *Angewandte Chemie International Edition* 56 (35): 10294–323. <https://doi.org/10.1002/anie.201611914>.
2. Naryshkin, N. A., M. Weetall, A. Dakka, J. Narasimhan, X. Zhao, Z. Feng, K. K. Y. Ling, et al. 2014. "SMN2 Splicing Modifiers Improve Motor Function and Longevity in Mice with Spinal Muscular Atrophy." *Science* 345 (6197): 688–93. <https://doi.org/10.1126/science.1250127>.
3. Sivaramakrishnan, Manaswini, Kathleen D. McCarthy, Sébastien Campagne, Sylwia Huber, Sonja Meier, Angélique Augustin, Tobias Heckel, et al. 2017. "Binding to SMN2 Pre-MRNA-Protein Complex Elicits Specificity for Small Molecule Splicing Modifiers." *Nature Communications* 8 (November): 1476. <https://doi.org/10.1038/s41467-017-01559-4>.
4. Ratni, Hasane, Martin Ebeling, John Baird, Stefanie Bendels, Johan Bylund, Karen S. Chen, Nora Denk, et al. 2018. "Discovery of Risdiplam, a Selective Survival of Motor Neuron-2 (SMN2) Gene Splicing Modifier for the Treatment of Spinal Muscular Atrophy (SMA)." *Journal of Medicinal Chemistry* 61 (15): 6501–17. <https://doi.org/10.1021/acs.jmedchem.8b00741>.
5. Hagedorn, Peter H., Malene Pontoppidan, Tina S. Bisgaard, Marco Berrera, Andreas Dieckmann, Martin Ebeling, Marianne R. Møller, et al. 2018. "Identifying and Avoiding Off-Target Effects of RNase H-Dependent Antisense Oligonucleotides in Mice." *Nucleic Acids Research* 46 (11): 5366–80. <https://doi.org/10.1093/nar/gky397>.
6. Ding, Yu, Yitian Fei, and Boxun Lu. 2020. "Emerging New Concepts of Degrader Technologies." *Trends in Pharmacological Sciences* 41 (7): 464–74. <https://doi.org/10.1016/j.tips.2020.04.005>.
7. Donovan, Katherine A., Fleur M. Ferguson, Jonathan W. Bushman, Nicholas A. Eleuteri, Debabrata Bhunia, SeongShick Ryu, Li Tan, et al. 2020. "Mapping the Degradable Kinome Provides a Resource for Expedited Degrader Development." *Cell* 183 (6): 1714–1731.e10. <https://doi.org/10.1016/j.cell.2020.10.038>.
8. Ottis, Philipp, Chiara Palladino, Phillip Thienger, Adrian Britschgi, Christian Heichinger, Marco Berrera, Alice Julien-Laferriere, et al. 2019. "Cellular Resistance Mechanisms to Targeted Protein Degradation Converge Toward Impairment of the Engaged Ubiquitin Transfer Pathway." *ACS Chemical Biology* 14 (10): 2215–23. <https://doi.org/10.1021/acscchembio.9b00525>.

References (continued)

9. Stanton, Benjamin Z., Emma J. Chory, and Gerald R. Crabtree. 2018. "Chemically Induced Proximity in Biology and Medicine." *Science* 359 (6380): eaao5902. <https://doi.org/10.1126/science.aao5902>.
10. Baran, Dror, M. Gabriele Pszolla, Gideon D. Lapidoth, Christoffer Norn, Orly Dym, Tamar Unger, Shira Albeck, Michael D. Tyka, and Sarel J. Fleishman. 2017. "Principles for Computational Design of Binding Antibodies." *Proceedings of the National Academy of Sciences* 114 (41): 10900–905. <https://doi.org/10.1073/pnas.1707171114>.
11. Jain, Tushar, Tingwan Sun, Stéphanie Durand, Amy Hall, Nga Rewa Houston, Juergen H. Nett, Beth Sharkey, et al. 2017. "Biophysical Properties of the Clinical-Stage Antibody Landscape." *Proceedings of the National Academy of Sciences* 114 (5): 944–49. <https://doi.org/10.1073/pnas.1616408114>.
12. Saka, Koichiro, Taro Kakuzaki, Shoichi Metsugi, Daiki Kashiwagi, Kenji Yoshida, Manabu Wada, Hiroyuki Tsunoda, and Reiji Teramoto. 2021. "Antibody Design Using LSTM Based Deep Generative Model from Phage Display Library for Affinity Maturation." *Scientific Reports* 11 (1): 5852. <https://doi.org/10.1038/s41598-021-85274-7>.
13. Shirai, Hiroki, Catherine Prades, Randi Vita, Paolo Marcatili, Bojana Popovic, Jianqing Xu, John P. Overington, et al. 2014. "Antibody Informatics for Drug Discovery." *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, Recent advances in molecular engineering of antibody*, 1844 (11): 2002–15. <https://doi.org/10.1016/j.bbapap.2014.07.006>.
14. Muttenthaler, Markus, Glenn F. King, David J. Adams, and Paul F. Alewood. 2021. "Trends in Peptide Drug Discovery." *Nature Reviews Drug Discovery* 20 (4): 309–25. <https://doi.org/10.1038/s41573-020-00135-8>.
15. Hagedorn, Peter H., Robert Persson, Erik D. Funder, Nanna Albæk, Sanna L. Diemer, Dennis J. Hansen, Marianne R. Møller, et al. 2018. "Locked Nucleic Acid: Modality, Diversity, and Drug Discovery." *Drug Discovery Today* 23 (1): 101–14. <https://doi.org/10.1016/j.drudis.2017.09.018>.
16. Matsui, Masayuki, and David R. Corey. 2017. "Non-Coding RNAs as Drug Targets." *Nature Reviews Drug Discovery* 16 (3): 167–79. <https://doi.org/10.1038/nrd.2016.117>.

References (continued)

17. Warner, Katherine Deigan, Christine E. Hajdin, and Kevin M. Weeks. 2018. "Principles for Targeting RNA with Drug-like Small Molecules." *Nature Reviews Drug Discovery* 17 (8): 547–58. <https://doi.org/10.1038/nrd.2018.93>.
18. Wang, Qiong, Yiqun Chen, Jaeyoung Park, Xiao Liu, Yifeng Hu, Tiexin Wang, Kevin McFarland, and Michael J. Betenbaugh. 2019. "Design and Production of Bispecific Antibodies." *Antibodies* 8 (3): 43. <https://doi.org/10.3390/antib8030043>.
19. Jensen, Karin J., Christian B. Moyer, and Kevin A. Janes. 2016. "Network Architecture Predisposes an Enzyme to Either Pharmacologic or Genetic Targeting." *Cell Systems* 2 (2): 112–21. <https://doi.org/10.1016/j.cels.2016.01.012>.
20. Suzuki, Masami, Chie Kato, and Atsuhiko Kato. 2015. "Therapeutic Antibodies: Their Mechanisms of Action and the Pathological Findings They Induce in Toxicity Studies." *Journal of Toxicologic Pathology* 28 (3): 133–39. <https://doi.org/10.1293/tox.2015-0031>.
21. Dammes, Niels, and Dan Peer. 2020. "Paving the Road for RNA Therapeutics." *Trends in Pharmacological Sciences* 41 (10): 755–75. <https://doi.org/10.1016/j.tips.2020.08.004>.
22. Levin, Arthur A. 2019. "Treating Disease at the RNA Level with Oligonucleotides." *New England Journal of Medicine* 380 (1): 57–70. <https://doi.org/10.1056/NEJMra1705346>.
23. Baranello, Giovanni, Basil T. Darras, John W. Day, Nicolas Deconinck, Andrea Klein, Riccardo Masson, Eugenio Mercuri, et al. 2021. "Risdiplam in Type 1 Spinal Muscular Atrophy." *New England Journal of Medicine* 384 (10): 915–23. <https://doi.org/10.1056/NEJMoa2009965>.
24. Ratni, Hasane, Martin Ebeling, John Baird, Stefanie Bendels, Johan Bylund, Karen S. Chen, Nora Denk, et al. 2018. "Discovery of Risdiplam, a Selective Survival of Motor Neuron-2 (SMN2) Gene Splicing Modifier for the Treatment of Spinal Muscular Atrophy (SMA)." *Journal of Medicinal Chemistry* 61 (15): 6501–17. <https://doi.org/10.1021/acs.jmedchem.8b00741>.
25. Sivaramakrishnan, Manaswini, Kathleen D. McCarthy, Sébastien Campagne, Sylwia Huber, Sonja Meier, Angélique Augustin, Tobias Heckel, et al. 2017. "Binding to SMN2 Pre-mRNA-Protein Complex Elicits Specificity for Small Molecule Splicing Modifiers." *Nature Communications* 8 (November): 1476. <https://doi.org/10.1038/s41467-017-01559-4>.
26. Singh, N. N., M. D. Howell, E. J. Androphy, and R. N. Singh. 2017. "How the Discovery of ISS-N1 Led to the First Medical Therapy for Spinal Muscular Atrophy." *Gene Therapy* 24 (9): 520–26. <https://doi.org/10.1038/gt.2017.34>.



References (continued)

27. Roberts, Thomas C., Robert Langer, and Matthew J. A. Wood. 2020. "Advances in Oligonucleotide Drug Delivery." *Nature Reviews Drug Discovery* 19 (10): 673–94. <https://doi.org/10.1038/s41573-020-0075-7>.
28. Tambuyzer, Erik, Benjamin Vandendriessche, Christopher P. Austin, Philip J. Brooks, Kristina Larsson, Katherine I. Miller Needleman, James Valentine, et al. 2020. "Therapies for Rare Diseases: Therapeutic Modalities, Progress and Challenges Ahead." *Nature Reviews Drug Discovery* 19 (2): 93–111. <https://doi.org/10.1038/s41573-019-0049-9>.
29. The Shape of Drugs to Come, Amgen, <https://www.amgenscience.com/features/the-shape-of-drugs-to-come/>
30. Citri, Ami, and Yosef Yarden. 2006. "EGF–ERBB Signalling: Towards the Systems Level." *Nature Reviews Molecular Cell Biology* 7 (7): 505–16. <https://doi.org/10.1038/nrm1962>.
31. SelleckChem tool compounds for the EGFR pathway, [https://www.selleckchem.com/EGFR\(HER\).html](https://www.selleckchem.com/EGFR(HER).html)
32. Zhang, M. May, Raman Bahal, Theodore P. Rasmussen, José E. Manautou, and Xiao-bo Zhong. 2021. "The Growth of SiRNA-Based Therapeutics: Updated Clinical Studies." *Biochemical Pharmacology*, January, 114432. <https://doi.org/10.1016/j.bcp.2021.114432>.
33. Wikipedia, RNA splicing, https://en.wikipedia.org/wiki/RNA_splicing
34. Wikipedia, RNA splicing, work by Agathman, used under CC-BY-3.0, https://commons.wikimedia.org/wiki/File:A_complex.jpg
35. Scotti, Marina M., and Maurice S. Swanson. 2016. "RNA Mis-Splicing in Disease." *Nature Reviews Genetics* 17 (1): 19–32. <https://doi.org/10.1038/nrg.2015.3>.
36. Jutzi, Daniel, Maureen V. Akinyi, Jonas Mechtersheimer, Mikko J. Frilander, and Marc-David Ruepp. 2018. "The Emerging Role of Minor Intron Splicing in Neurological Disorders." *Cell Stress* 2 (3): 40–54. <https://doi.org/10.15698/cst2018.03.126>.
37. Smith, C.I. Edvard, and Rula Zain. 2019. "Therapeutic Oligonucleotides: State of the Art." *Annual Review of Pharmacology and Toxicology* 59 (1): 605–30. <https://doi.org/10.1146/annurev-pharmtox-010818-021050>.
38. Fakhr, E., F. Zare, and L. Teimoori-Toolabi. 2016. "Precise and Efficient SiRNA Design: A Key Point in Competent Gene Silencing." *Cancer Gene Therapy* 23 (4): 73–82. <https://doi.org/10.1038/cgt.2016.4>.
39. Bennett, C. Frank, and Eric E. Swayze. 2010. "RNA Targeting Therapeutics: Molecular Mechanisms of Antisense Oligonucleotides as a Therapeutic Platform." *Annual Review of Pharmacology and Toxicology* 50 (1): 259–93. <https://doi.org/10.1146/annurev.pharmtox.010909.105654>.

References (continued)

40. [General policies for monoclonal antibodies. WHO](#)
41. Hammers, Christoph M., and John R. Stanley. 2014. "Antibody Phage Display: Technique and Applications." *The Journal of Investigative Dermatology* 134 (2): e17. <https://doi.org/10.1038/jid.2013.521>.
42. Alfaleh, Mohamed A., Hashem O. Alsaab, Ahmad Bakur Mahmoud, Almohanad A. Alkayyal, Martina L. Jones, Stephen M. Mahler, and Anwar M. Hashem. 2020. "Phage Display Derived Monoclonal Antibodies: From Bench to Bedside." *Frontiers in Immunology* 11. <https://doi.org/10.3389/fimmu.2020.01986>.
43. Hammers, Christoph M., and John R. Stanley. 2014. "Antibody Phage Display: Technique and Applications." *The Journal of Investigative Dermatology* 134 (2): e17. <https://doi.org/10.1038/jid.2013.521>.
44. V(D)J recombination, wikipedia, [https://en.wikipedia.org/wiki/V\(D\)J_recombination](https://en.wikipedia.org/wiki/V(D)J_recombination)
45. Jakobovits, Aya, Rafael G. Amado, Xiaodong Yang, Lorin Roskos, and Gisela Schwab. 2007. "From XenoMouse Technology to Panitumumab, the First Fully Human Antibody Product from Transgenic Mice." *Nature Biotechnology* 25 (10): 1134–43. <https://doi.org/10.1038/nbt1337>.
46. Rodríguez-Pérez, Fernando, and Michael Rape. 2018. "Unlocking a Dark Past." *eLife* 7 (September): e41002. <https://doi.org/10.7554/eLife.41002>.
47. CAR T-cell therapy, National Institute of Cancer, <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/car-t-cell-therapy>
48. Srivastava, Shivani, and Stanley R. Riddell. 2015. "Engineering CAR-T Cells: Design Concepts." *Trends in Immunology* 36 (8): 494–502. <https://doi.org/10.1016/j.it.2015.06.004>.
49. Waldman, Alex D., Jill M. Fritz, and Michael J. Lenardo. 2020. "A Guide to Cancer Immunotherapy: From T Cell Basic Science to Clinical Practice." *Nature Reviews Immunology* 20 (11): 651–68. <https://doi.org/10.1038/s41577-020-0306-5>.
50. Pollard, Andrew J., and Else M. Bijker. 2021. "A Guide to Vaccinology: From Basic Principles to New Developments." *Nature Reviews Immunology* 21 (2): 83–100. <https://doi.org/10.1038/s41577-020-00479-7>.
- 51.
- 52.
- 53.
- 54.

Supplementary Information

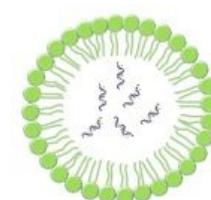
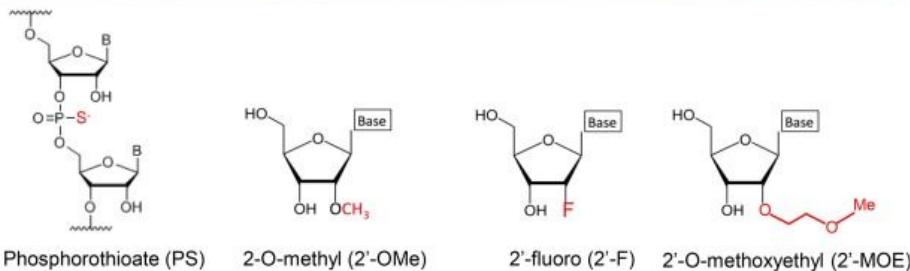
The growth of siRNA-based therapeutics

FDA-approved siRNA drugs	Patisiran	Givosiran	Lumasiran	
siRNA drugs in clinical trials	Vutrisiran	Nedosiran	Inclisiran	Fitusiran
	Teprasiran	Cosdosiran	Tivanisiran	
Drug	Alternative name	Company	Disease	Updated status
Patisiran	ONPATTRO	Alynlam	Hereditary transthyretin mediated amyloidosis	FDA approval in 10/08/2018 210922Orig1s000*
Givosiran	GIVLAARI	Alynlam	Acute hepatic porphyria	FDA approval in 11/20/2019 212194Orig1s000
Lumasiran	ALN-GO1	Alynlam	Primary hyperoxaluria type 1 (PH1)	FDA approval on 11/23/2020 214103Orig1s000
Vutrisiran	ALN-TTRsc02	Alynlam	Hereditary transthyretin mediated amyloidosis	Phase 3 trials ELIOS-A (NCT03759379)** HELIOS-B (NCT04153149)
Nedosiran	DCR-PHXC	Dicerna Alynlam	Primary hyperoxaluria	Phase 3 trial PHYOX 3 (NCT04042402)
Inclisiran	ALN-PCSSC	Alynlam Novartis	Hypercholesterolemia	Phase 3 trials ORION-9 (NCT03397121) ORION-10 (NCT03399370) ORION-11 (NCT03400800)
Fitusiran	ALN-AT3sc ALN-APC SAR439774	Alynlam Sanofi Genzyme	Hemophilia A and B	Phase 3 trials ATLAS-A/B (NCT03417245) ATLAS-INH (NCT03417102) ATLAS-PPX (NCT03549871) ATLAS-PEDS (NCT03974113) ATLAS-OLE (NCT03754790)
Teprasiran	AKII-5, DGF1, I-5NP, QPI-1002	Quark Novartis	Acute kidney injury Delayed graft function	Phase 3 trial ReGIFT (NCT02610296)
Cosdosiran	QPI-1007	Quark	Non-arteritic anterior ischemic optic neuropathy (NAION)	Phase 2/3 trial NCT02341560
Tivanisiran	SYL-1001	Sylentis	Dry eyes Ocular pain	Phase 3 trial HELIX (NCT03108664)

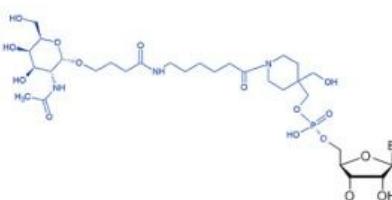
* FDA application number.

** ClinicalTrials.gov identifier number at <https://clinicaltrials.gov/ct2>

Drug	Backbone	Chemical modifications			Delivery platform
		PS	2'-OMe	2'-F	
Patisiran	-	+ (11)	-	-	LNP
Givosiran	+ (6)	+ (28)	+ (16)	-	GalNAc
Lumasiran	+ (6)	+ (34)	+ (10)	-	GalNAc
Vutrisiran	+ (6)	+ (35)	+ (9)	-	GalNAc
Nedosiran	+ (6)	+ (35)	+ (19)	-	GalNAc
Inclisiran	+ (6)	+ (32)	+ (11)	+ (1)	GalNAc
Fitusiran	+ (6)	+ (23)	+ (21)	-	GalNAc
Teprasiran	-	+ (19)	-	-	None
Cosdosiran	-	+ (9)	-	-	None
Tivanisiran	-	-	-	-	None

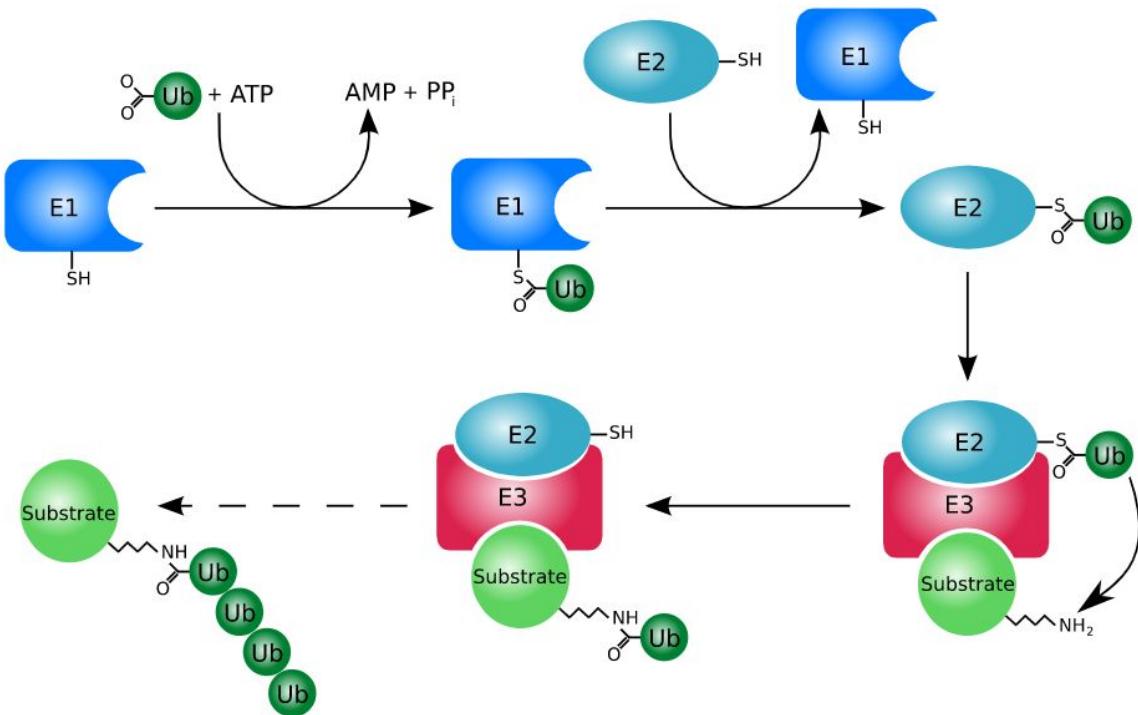
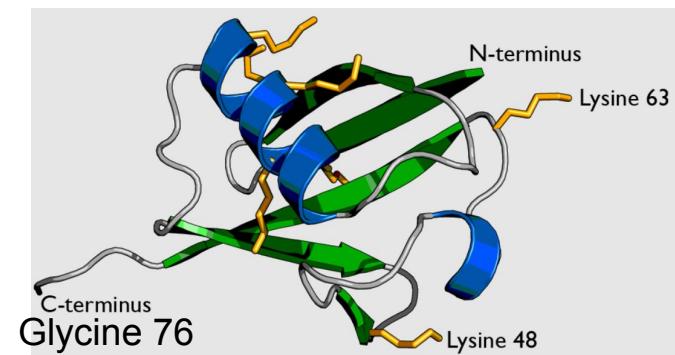


Lipid nanoparticle (LNP)



N-acetylgalactosamine (GalNAc)

Ubiquitination marks proteins to be degraded



work by [Rogerdodd](#), used under the CC-BY-SA 3.0 licence.